Add to the Body of Knowledge about Normal and Abnormal Biological Functions and Behavior

IN THIS SECTION:

SCIENCE ADVANCES | SCIENCE CAPSULES | STORIES OF DISCOVERY

SCIENCE ADVANCES

· The Fidelity of DNA Synthesis by Human DNA Polymerase ?, A Skin Cancer Susceptibility Gene Product
· Hidden Conformational Epitopes of HIV Envelope Protein Identified
· New Study Increases Understanding of Imprinting
· Explaining the Death of Insulin-Secreting Pancreatic Cells
· Controlling Appetite Through Triglyceride Metabolism
· New Insights Gained into Genetics and Treatment of Polycystic Kidney Disease
· Hematopoietic Stem Cells Pave Way to New Treatments for Disease
· Insights into the Role of the Immune System in the Development of Type 1 Diabetes
· New Understanding of Insulin Receptors and Insulin Resistance in Type 2 Diabetes
· Insights into the Molecular Mediators of Hyperglycemic Damage to Blood Vessels
· Research on Nuclear Receptors May Aid Drug Development
· Genomes of Yeast, Worm, and Fly Aid Understanding of Human Disease
· The Feasibility of Large-Scale Mutagenesis and Phenotyping Programs
· A Genetic Linkage Map of the Baboon Genome
· MRI Reveals Changes in Brain Structure Associated with Multiple Sclerosis
· Application of Laboratory Research to the Development of a Therapy for Chronic Myelogenous Leukemia
· Predicting Lung Cancer by Detecting Methylated Genes
· Gene is a Critical Player in Tumor Metastasis
· Anti-Apoptosis Gene is Overexpressed in Cancer Cells
· Clue from Inherited Childhood Disorder May Help Explain Breast Cancer
· Chemokines in Multiple Sclerosis: Prospects for Better Drugs
· Mouse Model of Neurofibromatosis Developed
· Understanding the Early Steps in Neurodegeneration
· How Nicotine Causes Long Lasting Effects on the Brain
· The Normal Development of the Olfactory System is Dependent on Neuronal Activity
· Rapid Progress in the Mapping and Cloning of Genes Responsible for Hereditary Hearing Impairment
· The Molecular Biology of Taste Signal Transduction: Diversity Personified
· Obese Mouse Reveals New Approach to Building Bone
· Mice Reveal a New Target for Prevention of Bone Loss
· Correlation Found Between Genetic Defect and Appearance of Skin Disease
· Both Embryonic and Aging-related Genes May Regulate the Aggressive Behavior of Joint Lining Cells in Rheumatoid Arthritis
· Two Related Proteins Form a Physical Complex with Calcineurin to Regulate Gene Expression of Muscle Fiber Types
· Genes Express Differences in Tendon and Ligament Repair
· Research Yields Clues About the Cycling of Hair
· A Neuroendocrine Model Explains Gender Differences in Behavioral Responses to Stress
· Building a Brain Synapse: Understanding the Axonal “HOV” Lane
· A Newly Discovered Protein Transports A Major Excitatory Brain Transmitter
· Single Neurons Play Complex Roles in Encoding Memories
· New Views On Brain Development
· A Scout’s Guide to Axon Guidance
· The Organization of Memories in the Hippocampus
· How Fear-Related Memories Are Stored – and Can Be Lost
· Experience and Biology Mold Capacity for Memory
· Imaging Shows the Brain is a Pictionary Plus

· Estorgen Increases Memory-Related Brain Cells in Adult Animals
· SLPI is Essential for Normal Wound Healing
· Cloning Resets the Telomere Clock in Cattle
· Genetically Mimicking Caloric Restriction Significantly Extends Yeast Life Span
· Use of Gene Expression Microarrays in Aging Research
· Extension of Average Life Span of Nematodes by Pharmacological Intervention
· Further Evidence that Presenilin-1 May Be One of the Major Amyloid-_ Forming Enzymes
· Neuropathology in Mice Expressing Mutant Tau Protein
· Transgenic Mice Expressing Human Alpha Synuclein have Motor Impairment
· A New Model of Parkinson's Disease
· Mad Cow Disease: The Cause of Human Fatal Neurodegenerative Disease?
· New Neurotrophic Factor for Brain Cholinergic Neurons
· Study of Genetic Recombination in Malaria Parasite Will Lead to Localization of Virulence and Drug-resistance Loci
· Spotlight on Visual Proteins: Visual Protein Sees the Light of Day
· Seeing with Rewired Brains
· Neovascularization Associated with Age-Related Macular Degeneration
· Function of Osteonectin/SPARC in the Retina
· Scientists Identify Malaria Gene that Confers Resistance to Chloroquine
· Determination of the Crystal Structure of a Natural Killer Cell Inhibitory Receptor Engaging a MHC Class I Molecule
· Crystal Structure of Novel Protein Reveals New Treatment Target for Immune-Mediated Diseases
· Genes Provide Clues to TB Persistence
· Researchers Identify Ebola Virus Gene that Causes Massive Hemorrhaging
· Snapshots of Nature: Crystal Structure of an Interaction that Triggers Asthma and Allergic Diseases
· Novel Protein on Dendritic Cells Delivers HIV to T Cells
· Resting $CD4^+$ T Cells are Not the Only Source of Resurgent HIV Virus Following HAART
· New Red Blood Cell Mutation Associated with Resistance to Malaria
· Sequencing the Mouse Genome: Providing Scientists with Tools to Interpret the Human Genome while Gaining Molecular Insight into a Powerful Model System
· Researchers Decipher the First Two Chapters of the Human Genetic Instruction Book
· Center For Inherited Disease Research: A Service to Help Researchers Identify Genes that Contribute to Human Disease
· Mammalian Gene Collection: A Resource for Studying Gene Expression and Function
· Early Childhood Stress Predicts Vulnerability to Alcoholism
· Alcoholic Fathers' Behavior Predicts Intellectual Deficits in Their Children
· Children's Emotional Response to Alcohol's Scent on Parents
· Understanding the Immune System of HIV-Infected Adolescents
· "Nerve Sprouting" May Be Useful Target for Preventing Heart Rhythm Disturbances
· Maternal Immune Tolerance of the Fetus
· Mutations Within a Skeletal Muscle Gene Cause Genetic Muscle Disease
· The Assembly of Neural Circuits
· Sex Hormones Provide Clues to Development of Autoimmunity
· Extent of Viral Diversity Early in Hepatitis C Virus Infection Predicts Whether the Infection will Become Chronic

· Clues to the Development of Alzheimer's Disease
· Vaults: Hollow Barrels or Treasure Chests
· Inhibitor Protein Works by Changing Shape
· Structural Basis of DNA Synthesis
· Genetic Basis Found for Hypertension During Pregnancy
· Possible Explanation Found for High Levels of Hypertension in Black Americans
· Understanding the Genetics of Pulmonary Fibrosis
· Research on Rare Disease has Implications for Understanding Cancer and Normal Cellular Processes
· Identification of Protein Responsible for Ebola's Devastating Effects Lays Groundwork for Vaccine Development
· DNA Repair – Integrated and Mulitstepped
· Influence of Thyroid Hormones on Fetal Brain
· Prostate Cancer Predictor – Mutated Androgen Metabolism Genes
· Prostate Cancer – New Model for Studying Early Initiating Events
· Killing Cancer Cells
· Insight into Down's Syndrome – Possible Role of ITSN Protein
· Increasing Production of White Blood Cells – New Line of Investigation
· Inhibiting the Inflammation Leading to Rheumatoid Arthritis and Crohn's Disease
· Identification of a Gastrointestinal Tumor Suppressor Gene
· Simple System Yields Clues About Anemia
· AIDS Therapy May Promote Diabetes
· Regulation of Protein Degradation
· Prenatal Androgen Exposure May Affect Insulin-Glucose Balance in Adulthood
· Zebrafish Gene Sheds Light on Embryonic Development of Blood Vessels
· Animal Models of Neurological Disorders
· Mapping of a Gene for Severe Pediatric Gastroesophageal Reflux
· Molecular Motor Activity is Regulated by Its Load
· Tissue Engineering Shows Promise for Articular Cartilage Repair
· Establishment of Water Barrier of Skin Requires Coordination of Events
· Novel Melanocyte Protein is Discovered
· Monoclonal Antibody Identifies Hair Follicle Stem Cells
· Role of Measles Virus in Paget's Disease of Bone Begins to Emerge
· Chondrocyte Migration May Enhance Repair of Damaged Cartilage
· Aggrecanase Plays a Role in Cartilage Destruction in Arthritic Diseases
· New Neutrophil Types Identified
· Exit Signs Help Traveling Neurons in the Brain
· Why Prostate Cancer Homes to Bone
· Genomic Approaches to Understanding Oral Cancer
· Mutation of PAX9 is Associated with Oligodontia
· Productive Human Immunodeficiency Virus-1 (HIV-1) Infection of Epithelial Cell Lines of Salivary Gland Origin
· Impaired Osteoclast Function Produces Osteopetrosis
· Cell Transplantation and Aging
· Mutations in the BRI Gene Result in the Deposition of Amyloid and Consequent Dementia
· Dietary Restriction Increases Neurotrophic Factor Production in the Brain and Thereby Protects

Neurons
· First Comparison of the Human Genome to a Complex Model Genome
· Data from the Human Genome Project Reveals Widespread Shuffling of Human DNA by LINE Elements
· Clue to the Mechanism of Latency in Ocular Herpes Infection
· New Images of a Major Lens Protein Give Insight into Protein Stability During Aging and Stress
· New Findings Help Explain How Patients with Glaucoma Lose Their Vision
· Development of Myopia
· The Genetic Basis of Stargardt Macular Dystrophy
· New Methods of Discovering Eye Tumor Genes
· The Nature of Activity in the Superior Colliculus
· The RPE65 Gene and the Visual Cycle
· Circadian Dependent Retinal Light Damage in Rats
· Discovery of Novel Mutation in BLNK Provides New Insight into B Cell Development
· Diesel Exhaust Particles (DEPs) Induce Allergic Antibody
· Discovery of New Pathway for Immune Recognition of Tuberculosis
· Genetic Mutation Linked to Missing Immunoregulatory Molecule
· Mutation Inhibits Proper Immune System Functioning
· Degradation of Defective Proteins Makes Antigens Available to Stimulate Immune Responses
· Identification of Protein Responsible for Persistence of Kaposi's Sarcoma-Associated Herpesvirus
· Discovering the Genetic Basis of Hearing Loss
· Longevity Genes
· Working With the Building Blocks of Life
· Elucidating the Role of Growth Factors in Embryonic Development
· Fruit Fly Genome Sequence will Provide Further Insights to Cancer and Aging
· Genetic Mutation Causes Common Defect in Early Development of Human Forebrain
· Cocaine High Related to How and How Quickly the Drug Reaches the Brain
· Marijuana Ingredient May Promote Tumor Growth
· Moderate Weight Loss OK for Overweight Moms Who Breast Feed
· Phospholamban Affects Contractility of the Heart
· Understanding Narcolepsy
· Pain Memories
· Obesity and Malalignment Correlate With Knee Osteoarthritis
· Exercise Results in Metabolic Shifts Lasting Several Days
· Molecular Mechanisms of Calcium Influx
· Changes in Physiological Response Characteristics of Cortical Neurons in Aging Primate Brain
· Changes in Diapered and Nondiapered Infant Skin
· Structural Characterization of Tear Components
· Plasma Membrane Cholesterol Release Modulates the Activation of Mammalian Sperm
· New Insights into the Natural History of Hepatitis C Virus Infection in Injection Drug Users
· Home Culture, Health Status Influence Infant Mortality Among U.S. Born Puerto Ricans
· Zidovudine Does Not Increase Adverse Pregnancy Outcomes
· Women's Fertile Days are Highly Unpredictable
· Psychosocial and Socioeconomic Factors Determine Who Stays in Long-Term Studies
· Presence of Rheumatoid Arthritis is Predictive of Development of Future Comorbidities

Alcohol Changes the Shape of Proteins

Background: Alcohol causes its behavioral effects by changing the function of nerve cells. For many years, it was assumed that alcohol causes intoxication by disrupting the nerve cell's surface membrane, which is composed of fatty molecules called "lipids." More recently, however, research has turned away from this picture of alcohol action and focused on alcohol's effects on proteins embedded in the lipids. Some of these proteins – "receptors" – transmit chemical messages from outside the cell into the cell, thereby integrating the function of the various cells in the body. Increasing evidence suggests that alcohol molecules bind to specific sites on receptors for neurotransmitters – molecules that function as chemical messengers in the brain – altering the activity of these receptors and, therefore, of cells.

A question remains, however, regarding the extent to which alcohol's impact on receptor and cell function is due to its interaction with the lipid molecules of the membrane itself, a more direct impact on proteins in the membrane, or both. Researchers in this study looked at the competition between alcohol and water in binding to cell membranes to find out if alcohol, by displacing water, could alter the structure of cell membranes or their receptors, thereby changing their function.

Finding: Scientists exposed membranes from the photoreceptor cells of cow retinas to alcohol and osmolytes, substances that cause shifts in the amount of water inside and outside cells. By measuring with a high degree of precision the interaction of alcohol and water with the lipid membrane and with the protein receptors associated with it, they found that alcohol molecules replaced some of the water molecules found on the surface of the light-sensitive receptor protein rhodopsin. (Rhodopsin is one of the best-understood proteins in a functional family that includes neurotransmitter receptors, making it an ideal tool for this research.) In replacing water, alcohol molecules were able to help induce a change in the structure of rhodopsin, thus enhancing its function. In addition, this change in rhodopsin structure was also aided by the action of alcohol on the lipid molecules of the membrane.

Implications: These findings are the first explicit demonstration that alcohol can change the structure and, thus, the activity of a protein receptor by altering water balance – a novel mechanism unrelated to neurotransmitter-receptor binding. The work also showed that alcohol does have a direct effect on membrane lipids, a change that also influences receptor activity. The scientists were able to measure the relative importance of these effects on receptor activity, showing that both are involved in alcohol's action in this system. Many receptors similar to rhodopsin are key components of the nervous system, through which alcohol causes its behavioral effects. Thus, the findings described here suggest that similar effects may be seen elsewhere in the nervous system and highlight the importance of this line of research for understanding alcohol's actions.

Mitchell DC, Litman BJ: Effect of ethanol and osmotic stress on receptor conformation reduced water amplifies the effect of ethanol on metarhodopsin II formation. The Journal of Biological Chemistry, 275(8):5355-60. 2000.

Scientists Close in on Alcohol's Suspected Binding Site by Putting it in Overdrive

Background: Proteins are key players in the nervous system. For example, they act as molecular "gates" that let certain substances in and out of nerve cells, maintaining the environments the cells need to function. These gates are part of complex protein structures--receptors--on the membrane surrounding the cell. Molecules that act as chemical signals dock onto the receptors, triggering chemical reactions necessary for normal function of the entire organism.

Among the substances needed for normal function in nerve cells are positively or negatively charged atoms ("ions"). Some molecular gates--ligand-gated ion channels or "LGICs"--in receptors respond to chemical signals that bind to them, "telling" them to let specific ions in or out of the nerve cell. The balance of positive and negative charges thus established by the cell temporarily enhances or supresses its ability to transmit electrical impulses. These activities are crucial to the function of the nervous system. Alcohol suppresses some of them, and a major question in alcohol research is where on nerve cells alcohol docks to trigger its effects.

Recently, scientists conducted studies to determine if alcohol's docking sites are in LGICs in two receptors, GABA? and glycine, that suppress electrical impulses in nerve cells. (Alcohol is known to cause even more suppression, via these receptors). By inserting substitutes for amino acids--building blocks of proteins--one at a time, they determined which of the amino acids had to be present in the LGICs for alcohol to bind and exert its effects. Next, researchers tested their hypothesis that these amino acids were pocket structures where alcohol molecules could fit. They modified alcohol molecules so that they were too long to fit into the suspected pocket and found that nerve cells normally suppressed by alcohol weren't affected by the modified molecules.

Because of alcohol's chemical make-up, its interactions with other molecules are fleeting, making activities like binding difficult to measure. Scientists recently modified the amino acids in question, *in vitro*, so that they would permanently bind with alcohol-like compounds when they docked, enabling researchers to measure the effect of the compounds' presence. They used LGICs in the GABA? and glycine receptors, whose suppressive effects are amplified by alcohol.

Advance: Researchers provided strong evidence that molecules of alcohol-like compounds bind to a single site in these receptors. Alcohol-like molecules docked permanently to the modified versions of the amino acids suspected of being the binding site for alcohol and anesthetics. The two receptors in which the LGICs containing these amino acids are located went into permanent suppression-overdrive when the alcohol-like molecules were thus irreversibly attached to them.

Implications: Alcohols' and anesthetics' modes of action are essentially the same, and implications of this study are significant for both. When scientists identify the docking sites they seek, they can design safer anesthetics that narrowly target the appropriate part of the nervous system, and medications that block alcohol's binding site very specifically,(unlike current medications), in people at high risk for alcoholism or who have become alcoholic.

Mascia MP, Trudell JR, Harris RA: Specific binding sites for alcohols and anesthetics on ligand-gated ion channels. Proceedings of the National Academy of Sciences, 97(16):9305-10. 2000.

A Mechanism of Alcoholic Liver Injury Identified

Background: Alcohol is unique among substances of abuse in a variety of ways. One of them is the far-reaching physical damage that alcohol causes to the body. For example, alcohol has the potential to destroy not only the brain, but also the liver and other tissues and organs.

For alcohol researchers, identifying the mechanisms by which alcohol injures the liver has been an ongoing challenge, and one to which they have responded with steady progress. For example, they know that alcohol promotes "leakage" from the bowel, into the bloodstream, of toxins associated with certain bacteria. These toxins travel through the blood to the liver, where they bind to membranes of Kupffer cells – a specialized type of liver cell. The binding of the toxin to the Kuppfer cell membrane acts as a signal for a series of damaging events to begin in the cell.

Scientists know some components of this alcohol-induced series of events in the cell. They know that the signal from the bowel-generated toxin stimulates Kupffer cells to produce free radicals – damaging, unstable molecules. These free radicals break apart a molecular complex that contains the substance "nuclear factor-?B" (NF-?B). Once NF-?B is freed from the molecular complex, it travels to the cell's nucleus, where genetic material that instructs the cell on what proteins to produce resides. Among them is the protein "tumor necrosis factor alpha" (TNF-a), which, in excess, causes injury. When NF-?B binds to the nucleus, the cell produces excess TNF-a, which invades the primary cells of the liver, causing damage.

One of the questions scientists still had concerned one of the steps at the beginning of this process, after the toxin has signaled Kupffer cells, but before free radicals have formed. Kupffer cells contain the compound xanthine oxidase, a potential source of free-radical formation. Is xanthine oxidase one of the sources of free radicals that cause alcohol-related liver damage? Scientists found out by giving alcohol-fed rats the compound allopurinol, which is known to inhibit xanthine oxidase and to disable free radicals by binding to them. The scientists compared these rats to others given alcohol, but not allopurinol, or given neither alcohol nor allopurinol.

Finding: Rats given alcohol had five times more liver damage than did rats not given alcohol. However, those also given allopurinol had 50 percent less damage. Allopurinol rats also had less of the inflammation, tissue death, and fatty deposits of alcoholic liver disease. Alcohol caused free radicals to form, but 40 percent less so in allopurinol rats. The increased, alcohol-induced binding of NF-?B to the nucleus – an initial step by which Kupffer cells cause tissue-damaging amounts of TNF-a to be produced – returned to normal in rats given allopurinol.

Implications: This finding is a powerful addition to the body of research suggesting that free radicals play a major role in alcohol-induced liver damage, and identifies one of the free radical systems involved. It is a key contribution to the search for mechanisms by which alcohol damages the liver and, ultimately, to the search for treatments.

Kono H, Rusyn I, Bradford BU, Connor HD, Mason RP, Thurman RG: Allopurinol prevents early alcohol-induced liver injury in rats. Journal of Pharmacology and Experimental Therapeutics, 293(1):296-303. 2000.

Potential for Preventing Alcohol's Damage to Fetal Development

Background: As command central for the nervous system, the brain regulates everything from movement and memory to the intellectual functions that make humans unique. Crucial to the developing nervous system and brain and to the development of tissues that make up the body's various structures is cell adhesion; that is, the ability of cells to attach to one another via special proteins on the cells' surfaces. "L-1" is one such protein. Ethanol – the kind of alcohol in beverages – inhibits this essential cell-to-cell attachment, suggesting a mechanism underlying the damage of fetal alcohol syndrome, one of the most prevalent forms of mental retardation in the United States and a cause of numerous behavioral abnormalities and physical malformations.

Evidence suggests that ethanol inhibits cell-to-cell adhesion by binding to specific pockets in the complex structures of proteins. A key task for scientists is to identify the structures of these pockets and to design pharmaceuticals that will bind to them, thus preventing ethanol from binding there and causing damage to the developing fetus.

Recently, scientists tested *in vitro* nerve cells and connective-tissue cells containing either L-1 or another protein, BMP-7, that triggers biological activities that lead to cell adhesion. Researchers already knew that ethanol inhibits L-1 and necessary developmental changes induced by BMP-7. Molecules of different kinds of alcohols differ in the lengths of chains of carbon atoms that comprise part of their chemical make-up. The researchers used a series of alcohols of differing carbon-chain lengths and shapes to determine what structures needed to be in place for alcohols to cause their pathologic inhibition of L1 cell adhesion.

Advance: In the *in vitro* tests described above, researchers found that alcohols containing chains of less than five carbon atoms, including ethanol, inhibited cell adhesion. However, alcohols with more than five carbon atoms in their chains did not inhibit cell adhesion, suggesting that their size prevented these alcohols' molecules from fitting into the protein pocket involved in regulating cell adhesion. When the scientists tested shorter-chain alcohols by attaching additional carbon-containing molecules to the alcohol's "backbone" carbon structure, thus forming branching shapes, the ability of the alcohol to inhibit L-1 binding again was altered. This indicates that it is the shape of the alcohol molecule, not the length of its chain, per se, that affects its pathologic ability to inhibit cell adhesion.

Implications: Agents that block ethanol's (beverage alcohol's) deleterious effects on cell adhesion and other developmental processes would provide clinicians with a valuable tool for preventing the harmful effects of maternal drinking on the fetus. The research described here is an important step in identifying the mechanisms involved in alcohol-related fetal damage and potential targets at which to direct pharmaceutical design.

Wilkemeyer MF, Sebastian AB, Smith SA, Charness ME: Antagonists of alcohol inhibition of cell adhesion. Proceedings of the National Academy of Science, 97(7):3690-5. 2000.

Targeting Cellular E-mail: Alcohol and Potassium Channels

Background: The effects of alcohol – intoxication and sedation – are a result of its interactions in the nervous system. A crucial task for alcohol researchers is to discover the many intricate biological mechanisms that alcohol triggers in nerve cells. Identifying these mechanisms will help scientists design more effective medications to prevent and treat alcoholism.

Scientists have long known that one of the key functions of nerve cells is to conduct electrical impulses – kind of a cellular e-mail system by which the nervous system transmits messages – and that charged particles of the mineral potassium are necessary for this to happen. Potassium channels are special conduits in nerve cells that transport these charged potassium particles in and out of the cells, thus changing the concentration of potassium within them. These intracellular changes in potassium concentration set up conditions that either enhance or inhibit electrical impulse conduction in nerve cells. When the concentration in the cell is higher, the cell is more electrically excitable; when the concentration drops, the cell's ability to carry electrical impulses is dampened. Scientists hypothesized that if alcohol acts on potassium channels, this could be at least one of the mechanisms through which it dampens the nervous system.

In general, scientists have found that alcohol does not affect the functioning of potassium channels. However, researchers recently examined a specific type of potassium channel, G-protein-coupled inwardly rectifying potassium channels, or "GIRKS," that yielded a different result. GIRKS are widespread in the brain, the command center of the nervous system. They are activated by neurotransmitters and receptors (chemical messages and the protein structures on nerve cells that receive them, respectively) and by other substances that transmit chemical signals within nerve cells.

Advance: In tests of *in vitro* nerve cells of the brain, researchers found that GIRK activity is enhanced by the same levels of alcohol that cause behavioral changes in humans. "Enhanced GIRK activity" means that the channels transported more potassium out of the cells, lowering the concentration of the charged particles within the cells, thus dampening their ability to carry electrical impulses.

Implications: Dampening of the nervous system is a hallmark of alcohol's behavioral effects. Identifying mechanisms that contribute to this and other effects of alcohol will enable scientists to direct their medication design at specific biological targets, thereby improving methods of preventing and treating alcoholism.

Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA: G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nature Neuroscience, 2(12):1084-90. 1999.

Gene Knockout Points to Receptor's Role in Alcoholism

Background: Alcohol interacts with the brain's nerve cells in a number of ways that make the experience of drinking feel pleasant for most people. For example, alcohol stimulates activity in proteins called "opioid receptors" that sit on the protective membranes that surround cells. When opioid receptors bind with certain protein fragments, opiate peptides, the result is the sedative and euphoric effect associated with opiate drugs. The binding of these peptides to opioid receptors are thought to be partly responsible for the reinforcing aspects of alcohol – the biologically driven desire to continue to drink – and to be among the physiologic reasons that people drink to the point of alcoholism.

Some medications that block the binding capacity of opioid receptors are moderately successful in reducing alcohol consumption in humans. However, opioid receptors exist in various subtypes, and among these medications' disadvantages is that they block the subtypes indiscriminately, rather than targeting the specific subtype that is involved in the effects of alcohol. If scientists can identify this subtype (or subtypes) of opioid receptor, their chances of designing medications that target that specific receptor, and of thereby reducing alcohol consumption with a minimum of side effects, will be improved.

In rodents bred to prefer alcohol, studies have shown that an opioid receptor subtype, the mu opioid receptor, appears in high numbers in areas of the brain suspected of being involved in the rewarding effects of drugs. This suggests a link between the mu receptor and propensity for alcohol use.

Advance: Scientists "knocked out" or disabled the gene that produces mu opioid receptors in mice and compared these animals to mice with the intact gene. The mice were placed in situations in which they could easily self-administer alcohol; they were also given the option of self-administering nonalcoholic fluids. In none of these situations did the mu knock-out mice self-administer alcohol, and in some experiments, they actually showed aversion to alcohol. Under the same conditions, the genetically normal mice did self-administer alcohol.

Implications: Once again, gene knockout techniques have provided scientists with the unique opportunity to isolate and test a biological factor that appears to contribute to alcoholism. The virtual obliteration of propensity to consume alcohol demonstrated in this research suggests the power of the mu receptor subtype in regulating drinking behavior. More importantly, this work indicates potential new directions for research on more effective medications for the treatment of alcoholism.

Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HWD, Koob GF, Gold LH: Mu opioid receptor knockout mice do not self-administer alcohol. Journal of Pharmacology and Experimental Therapeutics, 293(3):1002-8. 2000.

Neurosteroids: A Newly Recognized Avenue of Alcohol's Action

Background: Of prime importance to alcohol researchers is identification of the various mechanisms in the nervous system through which alcohol exerts its behavioral effects. Much recent research has focused on the interaction of alcohol with neurotransmitters – chemical messengers in the nervous system. Binding of neurotransmitters to receptor molecules on the membrane that surrounds nerve cells triggers cellular activities that underlie behavior.

Scientists now have evidence that neurosteroids – hormones that help regulate the functions of the nervous system – interact directly with the receptor molecules on the cell membrane to help regulate the pace of message transmission among nerve cells. Until recently, neurosteroids were thought to influence nervous system function by influencing gene expression – the production of proteins based on DNA blueprints contained in genes. Recognition of steroids' effects on receptors reflects a newly added dimension in scientists' understanding of them.

Different types of receptors play major roles in determining whether a nerve cell is excitable – likely to generate an electrical signal – or inhibited. One class of receptors, GABA, inhibits nerve-cell signaling as part of its normal function. The $GABA_A$ receptor is among them and is of particular interest to alcohol researchers. Several substances are known to influence the function of this $GABA_A$ receptor, thereby increasing or decreasing its inhibitory effects. Among these substances is the neurosteroid 3a,5a-THP. Studies have shown that alcohol's effects on the $GABA_A$ receptor are similar to those of 3a,5a-THP. Since the behavioral and neurochemical effects of alcohol and 3a,5a-THP on the $GABA_A$ receptor are so similar, scientists sought to discover if this neurosteroid is an intermediary of alcohol's action.

Advance: In rats, alcohol caused elevation of 3a,5a-THP levels in the brain, commensurate with the timing and amount of alcohol given. The amount of alcohol-related sedation the rats experienced correlated with their brain levels of 3a,5a-THP. Alcohol normally has anti-seizure properties, but when researchers gave the rats a substance, finasteride, that blocks production of 3a,5a-THP, alcohol's anti-seizure properties disappeared. In addition, scientists measured activity of nerve cells in the brain; as usual, alcohol dampened the cells' activity. However, administration of finasteride, which blocks synthesis of 3a,5a-THP, reversed this dampening.

Implications: These findings reveal that alcohol stimulates production of 3a,5a-THP in the brain and that 3a,5a-THP's interactions with alcohol contribute to alcohol's behavioral effects. The key to developing effective treatments for alcoholism is to identify the mechanisms through which alcohol acts on the nervous system and how these mechanisms fit together into neural circuits (networks of nerve cells that regulate specific activities in the brain). Scientists then can determine optimal points in these circuits at which to intervene. Newly identified elements in these processes add to the picture of alcohol's actions and provide new targets for intervention.

VanDoren MJ, Matthews CB, Janis GC, Grobin AC, Devaud LL, Morrow AL: Neuroactive steroid 3 a-hydroxy-5a-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. Journal of Neuroscience, 20(5):1982-9. 2000.

Mapping the Cocaine High Versus the Natural Reward High

Background: Researchers have known for many years that cocaine activates the brain's reward pathway in order to produce euphoria. This same pathway is activated by normal activities such as eating, drinking, and sex. Many of these naturally rewarding activities are also life sustaining. Considerable research efforts have focused on developing medications that can block cocaine's action on the reward pathway in order to treat cocaine addiction. One concern with this approach however, has been that medications that block cocaine's ability to produce feelings of reward might also interfere with reward from other normal activities. This presents a major challenge to researchers. If a medication were to prevent an individual from experiencing feelings of reward from normal activities so that they are unable to find anything pleasurable, it is unlikely that they would be willing to use the medication as a treatment for cocaine addiction. Consequently, researchers have been working to better characterize the reward pathway and the specific components of it that are impacted by cocaine. It is hoped that this better understanding will facilitate the development of a medication that can be used to treat cocaine addiction without interfering with the normal experience of pleasure.

Advance: Researchers have now shown that cocaine activates a part of the reward pathway that is separate from those components of the pathway that are activated by food and water. Using a relatively new technique, researchers were able to record the activity of nerve cells in response to cocaine, food, and water in a part of the reward pathway know as the nucleus accumbens. Specifically, they were able to determine how each individual nerve cell responded to the different types of rewarding activities. What they found was that the natural rewarding activities of ingesting food and water caused most neurons to respond in a similar pattern. In contrast, when cocaine was compared to food or water, very few neurons showed a similar pattern of response. These findings indicate that cocaine activates the reward pathway in a way that is separate and different from normal rewarding activities such as drinking and eating.

Implications: These results provide clear evidence that the components of the reward pathway that are activated by cocaine can be separated from those that are activated by normal rewarding activities. This exciting finding opens up the possibility of developing a medication for treating cocaine addiction that will interfere with cocaine's ability to produce reward without affecting other natural rewarding activities.

Carelli RM, Ijames SG, and Crumling AJ: Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus "natural" (water and food) reward. Journal of Neuroscience, 20(11):4255-66. 2000.

A Brain Chemical Found to Naturally Reduce Pain Responses

Background: In 1975 researchers first discovered that the brain itself produces chemicals called opiates that can naturally modulate pain. This research led to dramatic improvements in our understanding of what happens in the brain when someone is experiencing pain. In turn, this led to the development of new medications and treatments for pain. Despite these advances, the most effective treatments for severe pain are opiate drugs such as morphine that are less than ideal. Opiate drugs have a number of side effects that can be undesirable and even life threatening. Furthermore, their use in chronic pain remains limited by the development of tolerance and physical dependence. Consequently, considerable research efforts continue to focus on gaining an even greater understanding of the brain's role in the experience and alleviation of pain.

Advance: Studying pain response in rodents, researchers have now determined that a brain chemical, anandamide, produces a reduction in the perception of pain, or analgesia. Anandamide, which was first discovered in the 1990s, is chemically similar to tetrahydro-cannabinol (THC), the active ingredient in marijuana. Research has shown that it plays an important role in many brain functions. In this study, researchers showed that anandamide is naturally released in a specific region of the brain in response to painful stimulation and that this release results in analgesia. This pain reducing mechanism was also shown to be separate from and independent of the opiate analgesic pathways in that same brain region.

Implications: The discovery of a brain chemical that produces analgesia, but is unrelated to opiates, opens exciting new possibilities for the treatment of pain. By focusing specifically on the anandamide system, researchers may be able to develop medications that are at least as effective as opiates in the treatment of pain, but that do not have the side effects or potential for dependence of opiates. Also, since marijuana acts at the same brain sites as anandamide, further exploration of the anandamide system may lead to the development of medications to treat marijuana addiction.

Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC: Pain modulation by release of the endogenous cannabinoid anandamide. Proceedings of the National Academy of Sciences, 96(21):12198-12203. 1999.

The Role of Calcium in Establishing a Pregnancy

Background: A better understanding of the complex processes that aid cells in adhering to different surfaces is key to understanding how blastocysts successfully implant themselves into the uterine lining. (A blastocyst is a spherical layer of cells, with a small embryonic cell mass inside, that is formed after fertilization). Scientists know that the surface cells of tissues and organs are joined tightly together to protect themselves, as well as cells that lie beneath the surface layer, from mechanical damage, invasion by a foreign organism, and evaporation of fluid from the cells. To strengthen this protection, special membrane structures exist that join the cells together. These junctions are cemented with special proteins, one of which is sensitive to calcium. Calcium plays an important role in joining cells together. For instance, researchers have shown that even outside of the body, surface cells form junctions in culture dishes. When the concentration of calcium in the culture solution is reduced, the calcium-sensitive protein cannot function properly and, instead of dying, the cells separate.

Advance: Most recently, researchers demonstrated that mouse blastocysts which are in the process of implanting themselves into the uterine lining have specific receptors to molecules that appear on the uterine surface at the time of attachment. These molecules are named heparin-binding EGF-like growth factor (HB-EGF). The researchers also found that the coupling of the uterine HB-EGF with its receptor on the blastocyst surface stimulates the calcium channel of the implanting blastocyst. Opening this calcium channel increases the influx of calcium from the fluid trapped between the blastocyst and the small area of the uterine surface where it is attaching. This, in turn, reduces the calcium concentration surrounding the uterine surface cells, and weakens their junctions. As this happens, the uterine surface relaxes to aid "invasion" by the implanting blastocyst as it becomes imbedded in the lining.

Implications: These new findings support the concept that calcium plays an important role in supporting blastocyst implantation. The findings also help researchers to understand how the implanting blastocyst interacts with, and is accommodated by, the uterine surface as it becomes an embryo. Understanding the multiple factors involved in implantation could help researchers and clinicians find new ways to prevent certain types of infertility in women.

Wang J, Mayernik L, Schultz JF, Armant DR: Acceleration of trophoblast differentiation by heparin-binding EGF-like growth factor is dependent on the stage-specific activation of calcium influx by ErbB receptors in developing mouse blastocysts. Development, 127:33-44. 2000.

Bone Marrow Stem Cells Can Be Made to Differentiate into Neurons

Background: A central problem in treating neurological disorders is finding a source of new neurons, or "nerve cells," that can develop into functioning nerve tissue following brain or spinal cord injury or degeneration. Embryonic stem cells, which have the ability to grow into any type of cell found in the body, have been used to treat degenerative neurological conditions with some success, but use of these cells has raised some ethical questions. A potential way to overcome this problem is by using marrow stromal cells (MSCs). MSCs are a subclass of bone marrow stem cells that are capable of developing into bone, cartilage, and fat cells. When treated with certain chemicals, however, scientists believe that these cells can be prompted to develop into cells for other body tissues.

Advance: NIH grantees found that rodent and human MSCs can be stimulated to develop into neurons, raising hopes for new treatments for spinal cord injury, stroke, and degenerative brain diseases. Researchers made this finding by growing undifferentiated adult rat MSCs in culture for more than 20 cycles, which demonstrated their capacity to multiply rapidly. Then, by treating the cells with antioxidants and basic fibroblast growth factor, researchers caused the MSCs to convert into cells that resembled neurons and expressed neuron-specific proteins. Human MSCs treated with these chemicals also developed into neurons. Researchers are now transplanting the altered cells into various areas of the brain and spinal cord in rats, with preliminary data showing that the cells survive in the spinal cord for well over a month. In addition, the researchers are attempting to determine whether the cells can become integrated into an animal's nervous system.

Implications: While further studies need to be conducted in both animals and, ultimately, humans, MSCs may one day be useful for treating a wide variety of neurologic diseases. In addition, MSCs offer significant advantages over other stem cells because they are readily accessible, easily renewable, and avoid the risks of obtaining neural stem cells from deep within the brain. Furthermore, since MSCs are obtained from the patient's own body, researchers and clinicians can overcome the ethical as well as immunologic concerns associated with other sources of cells.

Woodbury D, Schwarz EJ, Prockop DJ, Black IB: Adult rat and human bone marrow stromal cells differentiate into neurons: Journal of Neuroscience Research, 61(4):364-70. 2000.

A Gene Involved in Learning

Background: The brain, which is the center of learning, is a unique organ designed to sense its internal and external environment, understand cause and effect relationships, and adapt in response to stimuli. To accomplish this task, a network of thousands of genes directs the development of a cellular network of one trillion neurons with 70 trillion connections among them. To add to such complexity, this neural network continually feeds back to the underlying gene network by regulating the expression of genes, which then alter the connections among the network neurons. This process originates early in development and creates a fairly crude pattern of "synaptic connections," or electrical impulses between nerve cells. Later, behavior and experiences promote the expansion, sharpening and refinement of these connections as synapses mature throughout later development and even during adulthood.

Advance: Using the fruit fly ("drosophila") model, which is particularly suited for genetic and behavioral analyses of learning, NIH-supported researchers studied the possible role of a novel learning gene, *latheo (lat),* in regulating the formation of different synapses ("synaptic plasticity"). The researchers found that LAT, the protein coded by the *lat* gene, is similar to a set of human nuclear proteins involved in DNA replication and appears to play a role in synaptic plasticity. In addition, they found that mutations in the *lat* gene result in reduced learning ability in adults, while stronger *lat* mutations are lethal during larval development. Thus, the LAT protein has an essential role during both larval development and adult learning. Based on these findings, researchers suggest that the *lat* gene becomes active when a specific experience, such as learning, stimulates neural activity. This activity, including that of *lat,* activates various signaling pathways among specific neurons. Continued experience, or learning, can sufficiently activate these signaling pathways to stimulate a "growth process" that ultimately changes synaptic structure and function. This growth process is associated with long-term memory function.

Implication: The work conducted by these researchers supports the theory that developmental plasticity (how nerve cells adapt as one matures) and long-term memory formation result from common underlying genetic mechanisms. Understanding the molecular mechanisms involved in developmental plasticity and long-term memory may eventually enhance knowledge regarding the molecular basis of brain disorders, including developmental learning disorders. Ultimately, this information may provide the basis for designing neurobiologically-based interventions to treat or modify these disorders.

Saitoe S, and Tully T: Making connections between developmental and behavioral plasticity in *Drosophila*. In J. McEachern & C. Shaw (Eds.) Toward a Theory of Neuroplasticity, Psychology Press, New York, (in press).

Rohrbough J, Pinto S, Mihalek RM, Tully T, and Broadie K: *latheo*, a *Drosophila* gene involved in learning regulates functional synaptic plasticity. Neuron, 23:55-70. 1999.

Abnormal Brain Pathways in Infants Who Die From Sudden Infant Death Syndrome

Background: About 3,000 infants in the United States succumbed to Sudden Infant Death Syndrome (SIDS) in 1997. SIDS is defined as the sudden death of an infant under one year of age that remains unexplained after a complete postmortem investigation. It occurs in an apparently healthy child during sleep. In recent years, scientists have learned that placing an infant to sleep on the back reduces the chance that a baby will die from SIDS. As a result, the number of SIDS deaths has declined by almost 40 percent as more and more care givers place infants to sleep on their backs. Although this is a welcome advance, SIDS cannot be prevented with absolute certainty, and there is no way to identify the infant who is likely to succumb to this condition. Thus, it is important that researchers learn why infants die of SIDS so that improved strategies can be developed to eliminate this devastating disorder.

Advance: NIH-supported researchers recently discovered that, compared with infants who die from other causes, many of the infants who died of SIDS had abnormalities in a major network of nerve cells in the brainstem. This network is believed to control respiration, blood pressure, temperature, and arousal during sleep. In the infants who succumb to SIDS, the binding of serotonin (a neurochemical that brain and nerve cells use to communicate) to receptors on the nerve cells in the network is greatly diminished.

Studies have shown that certain factors in a baby's sleep environment increase the chance that the baby will die from SIDS. These include sleeping on the stomach, sleeping on soft bedding, and having the face covered by bedding. Scientists theorize that these environments increase the chance that the baby will not get enough oxygen during sleep, will be exposed to too much carbon dioxide, or will overheat. These outcomes are potentially life threatening if the baby does not initiate the appropriate protective response, which is to wake up, take a breath, and move around. The serotonin network deficit could explain why these protective responses do not occur in many cases of SIDS.

Implications: SIDS is still the leading cause of death between one month to one year of age in the United States. This advance brings us closer to eliminating SIDS. Research can now focus on this particular neural network: how it develops and functions from fetal life through infancy, how to screen for newborns with the defective network, and how to manage these newborns clinically, and in the home environment, so that they are protected from an untimely death.

Panigrahy A, Filiano J, Sleeper LA, Frederick M, Valdes-Dapena M, Krous HF, Rava LA, Foley E, White WF, and Kinney HC: Decreased serotonergic receptor binding in rhombic lip-derived regions of the medulla oblongata in the sudden infant death syndrome. Journal of Neuropathology and Experimental Neurology, 59(5):377-84. 2000.

The Link Between Formula Additives and Children's Intelligence

Background: Human breast milk contains the essential fatty acids docosohexaenoic acid (DHA) and arachidonic acid (AA), which are believed to play a role in the development of the nervous system. While DHA and AA are routinely added to infant formula throughout Europe and Asia, these substances are not added to infant formulas in the U.S. Some studies have shown that breast-fed babies, regardless of socioeconomic status and the mother's education, perform better on cognitive tests than formula-fed babies, while other studies have been inconclusive or contradicted these results. Since previous studies on the link between breast milk and cognitive performance have been ambiguous, scientists have been trying to obtain definitive answers on whether breast feeding may indeed influence a child's intelligence.

Advance: Researchers measured the intelligence of 56 infants who were divided randomly into three groups. One group received formula supplemented with only DHA, while another received formula containing both DHA and AA. The control group received a commercial infant formula that did not contain either substance. All three groups of infants were enrolled in the study within five days of their birth and received one of the three formula types for 17 weeks. The infants were then assessed at 4 months and 12 months of age using two different visual acuity tests, which served as indirect measures of maturation in brain function. Researchers found that infants in the DHA/AA-supplemented groups performed significantly better in these tests.

The infants' overall intelligence and motor skills were later tested at 18 months using the 2nd edition of Bayley Scales of Infant Development (BSIDII), a standardized test used to measure early mental and psychomotor development. No differences were seen among the three groups on the Psychomotor Development Index of BSIDII, which measures motor skills, such as walking, jumping, and drawing. However, the infants differed significantly on the Mental Development Index (MDI) of the BSIDII, which measures young children's memory, problem-solving ability, and language capabilities. Infants whose formula was supplemented with both DHA and AA scored an average of 7 points higher on the Mental Development Index (MDI) compared to the control group; those receiving only DHA scored an average of 4 ½ points higher. These results suggest that early dietary DHA was a major determinant of improved performance on the MDI.

Implications: Whether DHA and AA should be added to infant formula in this country remains a pressing public health concern. The urgency of this problem has prompted the Food and Drug Administration to convene a panel of experts to review existing data and make a set of recommendations to the agency. The findings from this study serve as an important step in the comprehensive array of studies needed to determine whether DHA and AA should be added to infant formula.

Birch EE, Garfield S, Hoffman DR, Uauy RU, Birch DG: A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Developmental Medicine & Child Neurology, 42(3):174-81. 2000.

The Effects of Intensive Reading Instruction on Brain Function and Reading Behavior in Children

Background: Dyslexia, or the inability to read or understand what is being read, is estimated to affect 10 to 20 percent of children in the United States. It has been well-established that dyslexia is primarily a language-based disorder that is often caused by deficits in "phonological processing." Phonological processing refers to the ability to detect and manipulate sounds within speech units, such as spoken syllables and words. Deficits in the ability to segment words into constituent sounds lead to difficulties linking sounds to letters and letter patterns – a skill critical to learning to read an alphabetic written language. Previous research has demonstrated that dyslexia usually can be prevented if the disorder is identified by first grade and followed by intensive early reading intervention. However, correcting specific reading disabilities has been extremely difficult after children reach nine years of age. Scientists attribute this lack of response to an inability of the brain to respond and adapt to language and reading instruction after this age, and to a decrease in motivation to learn to read.

To address these issues, NIH-supported researchers designed intensive interventions for children ages 10 and older. Researchers wanted to determine whether a significant improvement in reading could be fostered if the treatment focused directly on developing the children's phonological language skills and word-reading skills. In addition, they conducted studies to determine whether improvements in reading behavior were associated with demonstrable changes in brain metabolic activity.

Advance: Working with children between 10 and 13 years of age with severe reading disability and family histories of multigenerational dyslexia, researchers reported that after these children participated in an intensive 30-hour reading and science workshop, they scored at or above grade level in reading skills. These skills were still maintained one year following the treatment in over 90 percent of the children studied. In addition, noninvasive neuroimaging studies conducted with the children before and after treatment indicated substantial changes in the brain's metabolic activity which were directly related to the behavioral changes in reading and language.

Implications: These discoveries indicate that reading disability in older children, previously thought to be unresponsive to treatment, *can* be corrected. Furthermore, these findings suggest that the improvement in reading is accompanied by significant changes in brain activity, indicating that the brain can adapt and possibly reorganize for language-related learning in middle childhood.

Richards TL, Corina D, Serafini S, et al: Effects of a phonologically driven treatment for dyslexia on lactate levels measured by proton MR spectroscopic imaging. American Journal of Neuroradiology, 21(5):916-22. 2000.

Rare Genetic Disease Sheds Light on Tumor Suppressor Gene

Background: Carney complex (CNC) is a rare genetic disease that results in multiple tumors of the heart, skin, breast, nervous system and endocrine glands (thyroid, pituitary, gonads, adrenal). CNC is also linked to abnormal skin pigmentation, similar to freckles, known as "lentigines." Recently, scientists have discovered a number of genes, known as proto-oncogenes, which are responsible for the regulation of cell growth or proliferation. Scientists have demonstrated that altering these genes can result both in tumor growth and in associated skin disorders, which are similar to those observed in CNC. The cause of these genetic mutations is unknown.

Because CNC affects many different organs of the body, scientists have speculated that the genes whose defects are responsible for CNC are normally involved in regulating basic cell functions common to all tissues and organs. Researchers hypothesized that by collecting data from the few families around the world that have CNC, and by using the tools of the Human Genome Project, they could identify the genes responsible for CNC and demonstrate that these genes are expressed in every cell.

Advance: In earlier studies, NIH scientists identified two chromosomal locations that potentially harbor the genes responsible for CNC. Recently, the search for the site on one of these chromosomes has been refined to a region where many other genes associated with tumor development are located; however, the specific CNC-causing gene has yet to be identified. In addition, the NIH scientists recently identified a gene on the other chromosome that is mutated in about half of all patients with CNC. Even more significantly, the scientists confirmed that this gene is expressed in almost all human cells and that it participates in the structure of one of the most important cellular communication pathways, that of protein kinase A. The gene directs the most common regulatory subunit of protein kinase A (PRKARIA) which, in the normal physiologic state, appears to act as a tumor suppressor. Therefore, when PRKARIA action is abolished, as in patients with CNC, tumors arise in various organs.

Implications: This new knowledge changes dramatically what was believed to be the function of protein kinase A and its involvement in tumor formation. Previously believed to be a tumor growth factor, PRKARIA now appears to be a tumor suppressor. Studies are now under way to screen patients with sporadic tumors for the presence of mutations of the PRKARIA gene. Eventually researchers hope that these findings can be used to develop drugs to treat patients with CNC and with other genetic and non-genetic tumors.

Kirschner LS, Carney JA, Pack S, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis C: Mutations of the gene encoding the protein kinase A type I-a regulatory subunit (PRKAR1A) in patients with the Carney Complex. Nature Genetics, 26(1):89-92. 2000.

Stratakis CA: Genetics of Carney complex and related familial lentiginoses, and other multiple tumor syndromes. Frontiers in Bioscience, 5:d353-66. 2000.

Promising Target for New Drugs Against Malaria

Background: Malaria is on the rise worldwide, killing at least one million individuals a year, mostly in Africa. More than half a billion people are infected with malaria worldwide and a quarter of the world's population is estimated to be at risk for this infection. Twenty-five percent or more of infected children die every year from malaria, one child succumbing every 12 seconds. The death rate among those infected is rising because the parasite, *Plasmodium falciparum*, which causes malaria is becoming resistant to conventional therapies. As of yet, there is no vaccine to prevent malaria infection and, until one is developed, there is an urgent need for new ways to treat the disease.

Advance: When the malaria parasite enters the body it attaches itself to the outer membrane of a red blood cell and slowly works its way into the cell. About 10 hours later, after the parasite has entered the cell, it needs a food source to sustain it and help it reproduce. NIH researchers have discovered that the malaria parasite, through the use of electrically charged particles, can create a pore-like hole through the red blood cell membrane. This channel acts like a straw through which the parasite supplies itself with the nutrients and elements that it needs for survival.

To demonstrate this phenomenon, the NIH researchers applied a microscopically thin glass electrode, with a diameter of about one $100{,}000^{th}$ of an inch, to the surfaces of infected and non-infected red blood cells and compared the electrical current flowing through the cell wall in response to an applied voltage (typically one tenth of a volt). Using special amplifiers, researchers were able to detect current changes that measured as little as a few picoamperes (one billionth of an ampere). With these instruments, the researchers also determined that red blood cells harboring the parasite had over a thousand such channels, which were much larger and had an electrical charge not associated with the normal pathways found in red blood cells.

Implications: Since these voltage-dependent channels are unique to infected red blood cells, they are probably a good target for new anti-malarial drugs. Investigators are now trying to determine if the parasite creates these channels by modifying a protein in the cell membrane of its host red blood cell, or by producing a new protein within the red blood cell that is then incorporated into the cell membrane. With this information, scientists could tailor drugs to cut off the parasite's supply lines, without affecting the ability of a normal blood cell to transport nutrients across its membrane.

Desai SA, Bezrukov SM, Zimmerberg J: A voltage-dependent channel involved in nutrient uptake by red blood cells infected with the malaria parasite. Nature, 406(6799):1001-5. 2000.

Biological Factor Found to Suppress Leukemia and Protect the Body from Infection

Background: Interferon consensus sequence binding protein (ICSBP) is a protein found in precursor blood cells (cells that give rise to blood cells). After cloning ICSBP, scientists produced a mouse that lacked ICSBP to learn more about how the protein functions. Among other things, the scientists learned that these mice developed a syndrome similar to chronic myelogenous leukemia, a type of cancer that affects the blood.

In addition, scientists found that the ICSBP-deficient mice were highly susceptible to various infections. One of the means that the body uses to protect itself from infection is with a relatively large cell called a macrophage. These cells play a fundamental role in protecting the body against infection by ingesting microorganisms that are foreign to the body and stimulating other infection-fighting cells to respond to injuries to the immune system. To carry out these protective activities, macrophages produce a critical protein called interleukin-12 (IL-12); without IL-12, the body is unable to effectively protect itself from infection.

Advance: NIH scientists have not only shown that ICSBP plays an important role in repressing myelogenous leukemia, but have demonstrated how this occurs. ICSBP regulates the growth of precursor blood cells and directs them to become macrophages. When the scientists inserted the ICSBP gene into the precursor blood cells of the ICSBP-deficient mouse, they found that the cells stopped growing uncontrollably, a hallmark of leukemia, and developed many macrophage functions. In essence, they showed that ICSBP both represses malignant growth and promotes macrophage development.

In addition, the scientists found that the ICSBP-deficient mice are susceptible to various infections mainly because their macrophages are unable to produce IL-12. Scientists have now learned that ICSBP also activates IL-12 production in macrophages.

Implications: This work confirms that ICSBP is a key factor in protecting the body against disease, and works by controlling the growth of impaired cells while increasing the number of infection-fighting cells. In addition, ICSBP activates IL-12 production in the macrophages. The macrophage needs IL-12 for the immune system to work effectively. With this new knowledge, scientists may be able to develop a treatment for leukemia and infectious disease by manipulating the ICSBP gene.

Tamura T, Nagamura-Inoue T, Shmeltzer Z, Kuwata T, Ozato K: ICSBP directs bipotential myeloid progenitor cells to differentiate into mature macrophages. Immunity, 13(2):155-65. 2000.

Wang I-M, Contursi C, Masumi A, Ma X, Trinchieri G, Ozato K: An IFN - ? - inducible transcription factor, IFN consensus sequence binding protein(ICSBP), stimulates IL-12 p40 expression in macrophages. Journal of Immunology, 165(1):271-9. 2000.

Herpes Virus Hijacks Cell's Own Transportation System

Background: Herpes is a major cause of infectious corneal blindness as well as a host of other diseases, ranging from the common cold sore to life-threatening brain inflammation. The disease is especially dangerous for infants and those with weakened immune systems. Symptoms include itching or burning skin at the infection site and blisters that become painful, oozing sores. After several days, the sores crust over and heal without leaving a scar. After the initial attack, the virus moves to nerve cells and remains there until set off again by a variety of factors, including fever, sun exposure, stress, and menstruation.

Advance: Scientists already know that the herpes virus finds its way from the nerve ending to the nerve cell body. This movement is critical for the virus to become dormant and to cause future flare-ups. A recent study suggests exactly how the virus manages this directed movement. A team of cell biologists conducted the study by injecting the herpes virus into the giant nerve cells of a squid. These squid cells are frequently used for such research because they are enormous – 7 centimeters (2.75 inches) long and almost a millimeter wide – about the size of a small, straightened-out paper clip. The scientists labeled the virus particles with a fluorescent protein and used a microscope and a digital camera to track the glowing virus as it moved up the giant neuron. The virus moved in one direction, and it traveled at the same constant speed that organelles move within cells. The researchers concluded that the virus usurps the nerve cells' own internal transport machinery.

Implications: Other research shows that herpes moves in the same direction and speed in rat nerve cells in culture. Together, these studies strongly suggest that the virus plays the same trick in humans. Understanding how the virus travels within nerve cells may lead to new treatments and perhaps cures for herpes infections. The work not only teaches us about herpes, but also about how normal cellular transport works.

Bearer EL, Breakefield XO, Schuback D, Reese TS, and LaVail JH: Retrograde axonal transport of herpes simplex virus: evidence for a single mechanism and a role for tegument. Proceedings of the National Academy of Sciences, 97(14):8146-50. 2000.

A New Target for Erectile Dysfunction Drugs

Background: Erectile dysfunction affects an estimated 10 to 15 million men in the United States. Erections are controlled primarily by a compound called nitric oxide (NO), a chemical that is normally released in response to sexual stimulation. NO rapidly relaxes the smooth muscles in the penis, allowing increased blood flow into the corpus cavernosum, a chamber of muscular, spongy tissue that is engorged with blood in the erect penis. Erectile dysfunction is usually caused by defects in the cascade of reactions that control this blood flow. One approach to treating the disorder is to use drugs to manipulate these chemical reactions. Viagra_, for example, enhances the smooth muscle relaxant effects of NO. Other chemicals in the cascade, including those that control the amount of available NO, remain appealing targets in the search for new drugs to treat the erectile dysfunction.

Advance: The amount of NO is regulated, in part, by an enzyme called arginase. A few years ago, a group of structural biologists used X-ray crystallography to solve the detailed, three-dimensional structure of arginase. Using this structure, the researchers designed a compound, called ABH, that blocks the action of arginase. In their new work, the scientists used strips of penile tissue from rabbits to prove that ABH causes relaxation of corpus cavernosum smooth muscle. This suggests that such an arginase-inhibiting compound may enhance human penile erections. The researchers also recently determined the detailed structure of ABH bound to arginase. Knowledge of how inhibitors such as ABH bind to arginase will enhance the ability of scientists to design other molecules – and perhaps marketable drugs – that block arginase.

Implications: Currently, there is only one oral medication approved to treat erectile dysfunction – Viagra_, which was approved for use in March 1998. The current research opens the door for the development of new drugs that target a different molecule involved in erection, arginase.

Cox JD, Kim NN, Traish AM, Christianson DW: Arginase-boronic acid complex highlights a physiological role in erectile function. Nature Structural Biology, 6(11):1043-47. 1999.

Synthetic Antibacterial Molecule Kills Drug-Resistant Bacteria

Background: Many previously treatable bacterial diseases have re-emerged with a vengeance, largely immune to penicillin and its close relatives. Drug-resistant bacteria are now a global health threat. In its June 2000 report entitled "Overcoming Antimicrobial Resistance," the World Health Organization stated that the growing resistance of major infectious organisms had reduced the healing power of "once life-saving medications to that of a sugar pill." The report highlighted several serious consequences, including an estimated 14,000 deaths in the United States.

Advance: Basic researchers have created a synthetic antibiotic molecule out of non-natural forms of amino acids called beta-amino acids. This "beta-peptide" mimics a class of natural antimicrobial molecules called magainins. These molecules exist in a wide variety of forms in nature and defend biological borders, such as skin, from invading bacteria. The beta-peptide has shown its antibiotic properties in the lab, killing both normal and drug-resistant strains of infectious bacteria, including a strain resistant to vancomycin – the "last resort" antibiotic that must be administered intravenously in a hospital.

Implications: The beta-peptide has two promising characteristics that distinguish it from traditional antibiotics. In combination, these two factors could be enough to prevent bacteria from developing resistance. First, researchers believe that bacteria may have trouble developing resistance to the beta-peptide because bacterial defenses may not recognize its non-natural amino acids. Second, the magainins that the beta-peptide mimics have been around for millions of years, yet bacteria have not become resistant to them. The beta-peptide must undergo further testing in the form of animal and clinical studies before its usefulness as a drug is known. But even if the beta-peptide never becomes a drug, this work is remarkable because it began as an effort to look at the folding properties of molecules, not to create an antibiotic. The research shows that it is possible to design from scratch the structure of a protein with a desired biological action.

Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH: Non-haemolytic beta-amino-acid oligomers. Nature, 404(6778): 565. 2000.

Key Enzyme Found Responsible for Abdominal Aortic Aneurysm

Background: Abdominal aortic aneurysm (AAA) is a bulging or ballooning of a weak area in the main artery, the aorta, as it runs from the heart down through the abdomen. AAAs are thought to occur through a complex interaction among various risk factors including atherosclerosis, aging, gender, cigarette smoking, and blood circulation factors, as well as through an undefined genetic component. Aneurysms tend to grow and can eventually rupture, causing profuse bleeding that usually results in death. Although scientists have long suspected that enzymes known as matrix metalloproteinases (MMPs) were responsible for weakening the aortic wall, they could not tell which MMP was primarily involved.

Advance: Scientists recently identified MMP-9 as the key enzyme responsible for development of AAAs, and showed that the antibiotic doxycycline impedes MMP-9 production. First, researchers initiated aortic wall injury in mice. AAAs subsequently developed in almost all of them, and inflammatory cells were found in the aneurysm area of the mice aortas that appeared to be producing several different MMPs. To determine whether the MMPs were contributing to aneurysm development, the researchers then initiated aortic wall injury in another group of mice in the same way as before, and treated those mice with the antibiotic doxycycline, which is known to restrain the production of MMPs. This time only 50 percent of the mice developed AAAs, supporting the idea that MMPs are involved in aneurysm development. To find out whether MMP-9 or the related MMP-12 is the primary enzyme in AAA development, the researchers next studied mice lacking one enzyme or both enzymes; results showed that MMP-9 is the key. Also, in a study in humans, the researchers analyzed aneurysm tissue removed from 15 patients during surgery. Those patients who had taken doxycycline before surgery had 5 times less MMP-9 production in their artery walls than those who had not taken the drug.

Implications: Up to 9 percent of individuals over the age of 65 are estimated to have an AAA. As the U.S. population continues to age, aneurysms are expected to affect more and more people. No drug treatment presently exists for preventing small aneurysms from developing into larger ones. Identification of MMP-9 as the key enzyme in AAA development might lead to new strategies for managing aneurysm development. Research results suggest that treatment with the antibiotic doxycycline has potential for preventing aneurysm growth in patients, thereby diminishing the need for expensive, risky surgery.

Pyo R, Lee JK, Shipley JM, et al: Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. The Journal of Clinical Investigation, 105(11):1641-9. 2000.

Curci JA, Mao D, Bohner DG, et al: Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. Journal of Vascular Surgery, 31(2):325-42. 2000.

Genetic Variation of ß2 Receptor Affects the Response to Asthma Treatment

Background: Asthma continues to be an increasingly important health issue in the United States and throughout the world. Despite the availability of several effective treatments, some asthma patients respond poorly to therapies that are highly effective for others. Several studies have suggested a genetic basis for this inability to respond to certain asthma therapies. Recently, considerable controversy has arisen over the role of bronchodilators, called beta agonists, in asthma treatment. Although bronchodilators are normally effective in opening airways and maintaining them in an open state, for some patients, evidence suggested that regular, scheduled use of beta agonists had a negative effect in controlling asthma. One explanation for this was a genetic variation.

Advance: New findings from the Asthma Clinical Research Network show that in certain asthma patients with a specific genotype, regularly scheduled use of bronchodilators worsens airflow in the lungs. Measuring morning and evening air flow, researchers determined that scheduled use of the beta agonists decreased patient air flow when compared with simply using the beta agonists on an "as needed" basis. The study suggested that a specific, detectable genetic variation is responsible for a slight alteration of the ß2 adrenergic receptor, a protein found in the lungs. When this altered version is present it causes a decreased response to beta agonists.

Implications: This finding is important because bronchodilators are the most commonly used asthma medication and many patients with mild asthma tend to increase the frequency of beta agonist use during exacerbations of symptoms. Understanding the genetics involved in response to asthma medication will allow physicians to better identify and treat those who might be prone to having no response or an adverse response. Understanding the complex genetics of asthma should aid in developing new approaches for primary and secondary prevention.

Israel E, Drazen JM, Liggett SB, *et al*: The effect of polymorphisms of the $ß_2$-adrenergic receptor on the response to regular use of Albuterol in asthma. American Journal of Respiratory and Critical Care Medicine, 162(1):75-80. 2000.

Improving Understanding of the Genetics of Lymphangioleiomyomatosis (LAM)

Background: Lymphangioleiomyomatosis (LAM) is a rare and devastating lung disease characterized by the overgrowth of smooth muscle-like cells in the lung. It primarily affects young women. Lung function worsens steadily due to overgrowth of the LAM cells and formation of numerous cysts throughout the lungs. LAM cases appear sporadically and do not seem to be inherited. However, a link has been observed between LAM and another, more common condition known as tuberous sclerosis complex (TSC), which is known to be inherited. Many women with TSC also have a mild form of LAM. Additionally, many women with LAM and TSC develop unusual, benign kidney tumors which contain typical LAM cells.

Advance: Researchers have discovered a genetic abnormality associated with LAM and TSC. The normal form of the gene codes for a protein that suppresses tumor formation. However, in cells from patients with LAM, both copies of the gene have been found to be defective.

Implications: There is no cure for LAM, although lung transplantation has helped some patients and anti-estrogen therapy may alleviate some of the symptoms. Understanding the genetic factors influencing LAM, as well as the pathogenesis of the disease, should aid in identifying new therapeutic targets and developing new treatments for this debilitating disease.

Carsillo T, Astrinidis A, Henske EP: Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proceedings of the National Academy of Sciences, 97(11):6085-90. 2000.

Antibodies Can Promote Blood Clotting in Autoimmune Diseases

Background: In autoimmune diseases such as systemic lupus erythematosus (SLE), the body produces antibodies that harm its own cells and tissues. Many patients with autoimmune diseases have antibodies that bind to prothrombin, a protein that is converted into another protein, called thrombin. Thrombin is required for blood clotting. It is formed when the requisite molecular bonds in prothrombin are broken. If the bonds in prothrombin cannot be broken, thrombin cannot be formed and the patient develops a bleeding disorder. Thus, according to conventional knowledge about interactions between antibodies and their targets, patients with SLE ought to develop bleeding disorders because the antibodies that impair prothrobin's conversion to thrombin would reduce the amount of thrombin available to form blood clots. Instead, patients with SLE develop complications from excessive clotting.

Advance: Researchers recently found an explanation for this paradox. Antibodies that bind to prothrombin actually promote the bond breakage that transforms it to thrombin, thereby promoting blood clotting instead of inhibiting it. The antibodies break bonds only in prothrombin and do not affect other proteins in the blood. Moreover, scientists identified antibody fragments that are capable of converting prothrombin to thrombin, and they determined which bonds are broken by the antibody fragments during the conversion.

Implications: The observation that patient antibodies are capable of converting prothrombin to thrombin provides an explanation for the excessive clotting observed in autoimmune diseases such as SLE and in other blood diseases including multiple myeloma. Identification of the specific molecular bonds that are broken in the course of the conversion and of antibody fragments that can stimulate the breakage provides a starting point for the development of therapies for excessive clotting in disorders in which such antibodies are produced.

Thiagarajan P, Dannenbring R, Matsuura K, Tramontano A, Gololobov G, Paul S: Monoclonal antibody light chain with prothrombinase activity. Biochemistry, 39(21):6459-65. 2000.

Severity of Symptoms and Risk of Mortality Due to Hypertrophic Cardiomyopathy Varies with Location and Type of Mutations

Background: Hypertrophic cardiomyopathy (HCM) – a thickening of the heart muscle that interferes with its pumping ability – has a prevalence of about 1 in 500-1000 in the United States. Although HCM frequently causes disabling symptoms, some patients with HCM are asymptomatic. In such cases, sudden cardiac death is the first symptom experienced. In fact, HCM is the most common cause of sudden cardiac death in apparently healthy young individuals such as athletes. At least 60 percent of HCM cases have a hereditary component.

Advance: Recently, researchers searching for the gene or genes involved in HCM identified several mutations that cause the disease. They also examined the clinical correlations of these different mutations, determining that the type of cardiac hypertrophy and clinical outcomes depend on the specific ways in which the mutations interfere with the heart contractile apparatus.

Implications: The genetic diagnoses afforded by understanding the clinical outcomes associated with these mutations will be valuable in the management of HCM. Specifically, such knowledge will contribute to predicting the severity of morbidity and the risk of mortality in individual patients, will help when deciding among various treatment options such as the implantation of defibrillators, and will aid with genetic counseling of prospective parents.

Olson TM, Doan TP, Kishimoto NY, Whitby FG, Ackerman MJ, Fananapazir L: Inherited and *de novo* mutations in cardiac actin gene cause hypertrophic cardiomyopathy. Journal of Molecular and Cellular Cardiology, 32(9):1687-94. 2000.

Human Pigmentation Disorder Linked to Genetic Defect in Inflammatory Pathway

Background: Incontinentia pigmenti (IP) is a genetic disorder characterized by unusual patterns of discolored skin. Males with this disorder usually die before birth, so females are the major patient group. In rare cases it can cause developmental abnormalities such as dwarfism and club foot. Although the skin abnormalities usually regress with age, in some individuals effects such as neurological problems and structural anamolies can persist throughout life. A team of researchers have identified genetic mutations in a critical component of the body's inflammation response as a key to understanding this disorder. They have also constructed a mouse model with this same genetic deficiency that mimics the human disease.

Advance: NF?B is a major regulator of immune responses stimulated by inflammatory stimuli such as tumor necrosis factor (TNFa), viruses, interleukin-1, and bacterial cell walls. NF?B also protects cells from TNFa stimulated programmed cell death. NF?B normally resides in the cytoplasm where it is bound by an inhibitory protein called I?B. During a pro-inflammatory stimulus, a chemical reaction occurs which leads to the breakdown of I?B and frees up NF?B so that it can move into the nucleus where it plays a role in the intricate translation of the genetic code into proteins. A group of researchers found skin lesions in female mice missing one of the IKK?/NEMO subunit of IKK. This skin condition proved to be remarkably similar to IP.

Both IP in humans and IKK?/NEMO deficiency in mice are conditions linked to the X chromosome. IKK?/NEMO deficiency in male mice leads to death around the twelfth day of gestation. Most human males with IP die before or soon after birth. IP in females is manifested by a blistering skin condition that eventually leaves the skin with pale streaks contrasted by areas of excessive pigmentation. The similarities in the two conditions prompted this team of investigators to look for the IKK subunits in biopsies and fibroblasts of IP patients. As in the mice, female human tissue proved markedly deficient in IKK?/NEMO. A male fetus and newborn whose mother had a positive diagnosis for IP were completely lacking IKK?/NEMO.

Implications: This study definitively links incontinentia pigmenti with deficiency of IKK?/NEMO production. This connection provides additional evidence for the importance of the IKK complex and NF?B for prevention of programmed cell death in mice and in humans. IKK?/NEMO deficient mice can be used as a model for studying the human disease IP and women with IP will be able to make more informed reproductive decisions.

Makris C, Godfrey VL, Krahn-Senftleben G, et al: Female mice heterozygous for IKK?/NEMO deficiencies develop a dermatopathy similar to the human X-linked disorder incontinentia pigmenti. Molecular Cell, 5(6):969-79. 2000.

Environmental Response Gene Found to Have Important Role in Fetal Development

Background: The past eighty years have seen an explosion in the number of synthetic chemicals produced and released into our environment. The human body has a vast system of metabolizing enzymes that are able to recognize these chemicals, break them down into more water-soluble forms, and excrete them from the body. Although scientists discovered many of these metabolic systems when looking for how the body handles manmade environmental toxicants, clearly these systems evolved for other important reasons.

One major environmental response gene is the one coding for the aryl hydrocarbon receptor (AHR). This receptor regulates the body's responses to environmental contaminants such as polycyclic aromatic hydrocarbons found in cigarette smoke, polychlorinated dioxins that contaminate industrial chemicals, and the wartime defoliant Agent Orange. Because of its role in mediating responses to environmental contaminants, the biology of the AHR has been extensively characterized from a toxicological viewpoint. New research, though, reveals that AHR also has an important role in vertebrate development.

Advance: Using a mouse model that completely lacked the gene coding for AHR, researchers showed that this gene plays an important role in regulating vascular, or blood vessel, growth during development. Mice lacking this gene were unable to develop mature blood supply structures, thus having an adverse effect on development of the liver, eye, and kidney.

Implications: This study shows that the AHR plays a role in the resolution of a number of vascular, or blood supply, structures. This aspect of vascular development has been referred to as "pruning," a process by which early vessels are removed to form a more mature vascular pattern. In mice lacking the AHR gene, this maturation does not take place. There are clinical implications for these results since there are congenital anomolies associated with defects in vascular development. These data also show that toxicologists need to investigate vascular endpoints when studying the toxicity of compounds that bind to the AHR.

Lahvis GP, Lindell SL, Thomas RS, et al.: Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. Proceedings of the National Academy of Sciences, 97(19):10442-47. 2000.

Insight into Development of Important Immune System Components

Background: The genetic material that controls all biologic functions is located in the nucleus of each cell. Initiating any biologic event requires that a specific part of this genetic material be "turned on" or transcribed. This initiation begins when specific natural or synthetic small molecules, referred to as ligands, bind to specific proteins in the nucleus that, much like a key in a lock, can fit into the receptor and unlock its power to communicate with a gene and initiate transcription.

Some years ago an increasing number of nuclear receptors were discovered. Because their ligands are unknown, they are referred to as "orphan" receptors and scientists are only now beginning to define the biological roles they play. Study of one such orphan receptor has recently revealed its critical role in the development and maintenance of immune system components.

Advance: Scientists investigated the retinoid-related orphan receptor (RORgamma). They constructed a mouse model that lacked the gene for this receptor. These mice were found to lack several important parts of the immune system, specifically peripheral and mesenteric lymph nodes as well a Peyer's patches. Lymph nodes are the part of the immune system that are responsible for storing and releasing white blood cells and for removing foreign materials from the lymph fluid. Peyer's patches are located in the small intestine and are responsible for destroying the bacteria that proliferate in this area.

The thymus was also found to be deficient, producing fewer thymocytes than normal mice. The data indicate that RORgamma has a role in regulating cell death (apoptosis) in thymocytes.

These findings demonstrate that the RORgamma orphan receptor plays a critical role in the development of the lymph nodes and in regulating thymus activity.

Implications: The thymus gland is a vital part of the immune system, releasing T-cells to fight outside invaders. It plays an important role early in life in development of immune system responsiveness. After puberty it shrinks in size and is replaced by fat. It has been thought to be relatively unimportant in adulthood. Recently, however, it has been discovered that people with the Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), can compensate for loss of T cells from this infection by "reawakening" the thymus to produce more T cells. This reported study gives valuable insight into the possible ways of manipulating the thymus into adulthood, as well as better understanding its critical role in early development of the immune system.

Kurebayashi S, Ueda E, Sakaue M, et al: Retinoid-related orphan receptor ? (ROR?) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. Proceedings of the National Academy of Sciences, 97(18):10132-37. 2000.

Fixing the Damage Done: Atomic Structure of a DNA Repair Enzyme

Background: The genetic material of all cells is under constant bombardment from environmental agents such as chemical carcinogens and physical agents like sunlight. This DNA damage, if not repaired, can lead to mutations and cancer. DNA repair pathways are highly conserved from bacteria to man. DNA repair is mediated by a multi-protein machine that surveys the contours of DNA looking for specific lesions. NIH researchers have solved the atomic structure of a bacterial DNA repair enzyme. This enzyme shares significant similarity with several human repair proteins. The protein structure resembles that of a large super family of proteins, called helicases.

Advance: The researchers overexpressed and purified a protein, UvrB, from a bacteria which normally grows at high temperatures. This temperature stability allowed crystals of the protein to form and to be used in X-ray crystallography studies. The authors were then able to solve the atomic structure of the UvrB protein. This is the first structure of a DNA repair protein from the DNA repair pathway that removes bulky DNA lesions such as those resulting from a wide variety of chemicals and physical agents such as food mutagens and sunlight. UvrB is folded in a similar manner as proteins of the superfamily called DNA helicases. DNA helicases are proteins that unwind the two strands of DNA. These scientists suggest that UvrB has adapted a helicase-like function to open up the two strands of the helix looking for specific damaged sites.

Implications: There are several cancer-prone diseases in humans caused by a deficiency in DNA repair enzymes. One syndrome called xeroderma pigmentosum (XP) is characterized by a 3,000 fold increase in skin cancer. There are eight know variants of this disease and one form, XP-D encodes a mutated form of a gene that shares significant functional homology to UvrB. Analysis of the amino acid sequences of the two proteins reveals highly conserved helicase motifs with 100% amino acid identify over long tracks of the protein. It is strongly believed that XP-D and UvrB will be folded into very similar three dimensional structures. Thus having the structure of UvrB will help inform how mutations in the XP-D protein can lead to a loss of DNA repair and an increased susceptibility to cancer.

Theis K, Chen PJ, Skorvaga M, Van Houten B, Kisker C: Crystal structure of UvrB, a DNA helicase adapted for nucleotide excision repair. Journal of the European Molecular Biology Organization, 18(24):6899-6907. 2000.

New Subfamily of Environmental Response Enzymes Discovered

Background: Nature has provided a vast family of enzymes that convert both synthetic and natural compounds to more polar, or water-soluble, chemicals. These cytochrome P450 enzymes have been extensively studied for their ability to act on environmental compounds. Increasingly we are becoming aware of their importance in the activation of endogenous compounds such as steroids, bile acids, and fatty acids. The P450 family of enzymes is clearly important for survival; these enzymes are ubiquitous in all living organisms and have been found in bacteria, yeast, plants and animals. A new subfamily has just been identified in a species known as killfish, which has similarities to mammalian enzyme systems.

Advance: Researchers have identified two new proteins that represent a novel subfamily of cytochrome P450s, the CYP2Ns. Both CYP2N1 and CYP2N2 are catalytically distinct enzymes that are active in the metabolism of environmental compounds, as well as the metabolism of the prostaglandin precursor, arachidonic acid. This subfamily appears to encode early vertebrate forms of arachidonic acid catalysts that are widely found in liver tissues and are regulated by environmental factors.

Implications: The importance of P450 enzymes as environmental response agents in mammals has been well established. Science has only recently begun to discover the natural role of these enzymes in the metabolism of endogenous chemicals. By studying early evolutionary variants of this important family of enzymes, scientists can better understand the natural functions of this class and can more readily predict how environmental agents can perturb biologic functions.

Oleksiak MF, Wu S, Parker C, Karchner SI, Stegeman, JJ, Zeldin DC: Identification, functional characterization and regulation of a new cytochrome P450 subfamily, the CYP2Ns. The Journal of Biological Chemistry, 275(4):2312-21. 2000.

Postnatal Sex Reversal of Ovaries – Insights into Estrogen Receptors

Background: Estrogen is a potent regulator of many cellular events in both men and women. The first step in this regulation involves the binding of estrogen to estrogen receptors. Currently two different estrogen receptors have been identified – the a and the ß. In order to identify the biologic roles and importance of these receptors, scientists have constructed mouse models in which these receptors have been eliminated. A new model deficient in both of these receptors is providing important insight into the role of estrogen in guiding reproductive tract development in mammals.

Advance: This new Estrogen Receptor Knockout (ERKO) mouse lacks both the a and ß forms. The males have normal appearing reproductive tracts, although the testes are affected and numbers and motility of sperm is reduced. Female mice lacking these estrogen receptors had all the normal reproductive tract components. As the mice matured, however, differences appeared. The most startling was in the ovaries. Female adult mice developed structures resembling seminiferous tubules of male testis. This finding is in marked contrast to the ovaries of mice lacking only one of the estrogen receptors.

Certain characteristics of this apparent sex reversal in adult mice indicates a redifferentiation of ovarian components rather than a developmental phenomenon. These characteristics include (1) the absence of these structure in the ovaries prior to puberty, (2) the consistent spherical shape of the "tubules," indicating they arise from a once healthy follicle, and (3) the fact that the affected area increases with age.

Implications: Normal reproductive tract development and the maintenance and proper differentiation state of ovarian cells requires both the a and the ß estrogen receptors. Clearly any environmental disturbances in either of these systems, even into early adulthood, can have adverse effects.

Couse JF, Hewitt SC, Bunch DO, Sar, M, Walker VR, Davis BJ, Korach KS: Postnatal sex reversal of the ovaries in mice lacking estrogen receptors a and_ß. Science, 286(5448):2328-31. 2000.

Glutathione: A Real "Knock-Out" for Mammalian Development

Background: Glutathione (GSH) is an essential intermediate in many physiologic reactions and functions. It metabolizes carcinogens and synthetic compounds and protects cells from free radical damage by adding sulfur atoms to the compounds which changes their chemical structures. Previous work has shown that newborn rats (but not adults) and guinea pigs which lack the ability to synthesize Vitamin C (ascorbate), die with widespread intracellular damage when a GSH inhibitor is administered. This damage occurs in intracellular organelles called mitochondria that are the "power factories" of cells and are a major source of free radicals in the form of reactive oxygen. The role GSH plays in growth and development has been difficult to study because GSH inhibitors given to pregnant rodents do not always completely inhibit GSH production.

Advance: To further investigate the role of GSH in development, this team of investigators used molecular genetics techniques to create a "knock-out" mouse unable to produce a key enzyme required for GSH synthesis rendering its cells incapable of producing GSH. Embryos lacking the genes necessary for GSH production undergo arrested development and die prior to day 8.5 of gestation. Death results from program cell death or apoptosis but not from a lack of cell growth or division. *In vitro* studies performed on cells harvested from embryos earlier in development thrive if supplemented with GSH or dithiothreitol, another source of sulfur atoms. However, these cells could not be rescued with any other reducing agents, or vitamin C or vitamin E.. Using electron microscopy, the investigators found no changes in the appearance of these cells' mitochondria.

Implications: These experiments demonstrate that GSH is required for mammalian development but that it is not necessary in cell culture. This is a stunning result since GSH is required for the breakdown of hydrogen peroxide by glutathione peroxide in the mitochondria. These results demonstrate that mitochondria must have other metabolic pathways for the break down of hydrogen peroxide, a normal by product of oxidative phosphorylation. According to the investigators, "the availability of these mutant cells deficient in GSH synthesis will allow further exploration of the role of cellular redox status in signal transduction, posttranslational regulation of proteins . . ., cell growth, differentiation, and cell death."

Shi ZZ, Osei-Frimpong J, Kala G, et al: Glutathione synthesis is essential for mouse development but not for cell growth in culture. Proceedings of the National Academy of Sciences, 97(10):5101-6. 2000.

Control of Mitochondrial Iron Metabolism by Products of the Iron-Sulfur Gene Complex

Background: A variety of enzymes contain iron-sulfur complexes that are critical for proper enzyme functioning and in some cases as regulatory switches. Examples of these enzymes are found in the tricarboxylic acid cycle, nitrogen fixation, and other important biochemical reactions. The most thorough characterization of the formation and maintenance of these clusters has been done in bacteria. Other work in yeast found similar proteins. These investigators studied two yeast proteins, Isa1p and Isa2p, that are products of the iron-sulfur complex gene cluster and are responsible for mitochondrial iron metabolism and formation and repair of iron-sulfur centers. Mitochondria are the energy-producing components of cells and are a major source of DNA-damaging free radicals in the form of reactive oxygen.

Advance: To test the function of Isa1p and Isa2p, disruptions in the genes coding for the two proteins were performed. The resulting mutant yeast lived but were dependent on lysine and glutamate for growth and exhibited a reduced capacity for energy production due to the accumulation of mutations in the mitochodrial DNA. The mutants also had very high mitochondrial iron concentrations and the activities of the iron-sulfur containing enzymes aconitase and succinate dehydrogenase were significantly reduced.

Implications: These results provide evidence that Isa1p and Isa2p play important roles in iron metabolism and the formation and maintenance of iron-sulfur enzyme complexes. The high mitochondrial iron concentration seen in the mutants causes DNA damage which is manifested in mitchondrial DNA mutations. Further understanding the metabolism of iron in mitochondria may have important implications for human diseases such as hemachromatosis and degenerative diseases.

Jensen LT, Culotta VC: Role of *Saccharomyces cerevisiae* ISA1 and ISA2 in iron homeostasis. Molecular and Cellular Biology, 20(11):3918-3927. 2000.

Folic Acid Binding Protein is Crucial for Mother to Fetus Folate Transfer

Background: Over the past 10-15 years there have been many news accounts about severe neurological birth defects along the U.S./Mexico border. The defects are characterized by improper or arrested growth and development of the brain and other neural tube defects. In some cases, the fetuses were partially or completely anencephalic with absolutely no chance of survival.

Research sponsored by NIH discovered folic acid deficiency as the major cause of this condition. Supplementation greatly reduced the occurrence of neural tube defects, but the fact that not all women enjoy the same protective effects from folic acid supplementation suggests that underlying genetic and environmental factors may be involved. Efforts to understand the complex gene/nutrient interactions underlying neural tube defect development have progressed slowly with most work focusing on the genes associated with the folic acid biosynthetic pathway or with transport into target cells.

Advance: To study the importance of the folic acid binding protein on the transport of folic acid into cells and its subsequent involvement in neural tube defects, researchers from Texas and Nebraska created a strain of transgenic mice in which the folic acid binding protein gene was inactivated. Using simple Mendelian genetics, they crossed animals that had an active and inactive copy of the gene which produced some fetuses that had only inactivated genes. In other words, these fetal mice pups had no genes for folic acid binding protein which made them incapable of processing folic acid normally. These fetuses showed severe neural tube defects. They died *in utero* at about day 8 of gestation and were resorbed. Further experiments in which rats with the same genetic makeup were given folic acid supplementation did produce live pups but neural tube defects were still present in some pups.

Implications: These experiments add additional scientific evidence of the important role folic acid plays in the development of the nervous system in mammals. Mice without genes for folic acid binding protein were all severely growth retarded and had severe malformations. All biochemical reactions that are folate dependent have yet to be elucidated. This new transgenic mouse strain will be a valuable tool for studying how the receptor is able to provide folate in sufficient quantities to sustain normal development.

Piedrahita JA, Oetama B, Bennett GD, et al: Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. Nature Genetics, 23(2):228-232. 1999.

Rendering the Brain More Vulnerable to Environmental Damage

Background: Dopamine is a chemical responsible for transmitting messages among the nerve cells of the brain. Disruption of this "neurotransmission" underlies such diverse conditions as Parkinson's Disease, schizophrenia, and Tourette syndrome. Dopamine relies on two transporter proteins to allow it to move into and out of cells. They are the plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2). DAT stops the action of dopamine by rapidly binding it and removing it. VMAT2 moves intracellular dopamine into vesicles for storage and later release. Recent data suggests that disturbing the tightly controlled balance of these two transporters makes nerve cells vulnerable to damage by a variety of environmental insults including cocaine and organochlorine insecticides such as dieldrin.

Advance: In this article, the authors review recent work which describes the control of DAT and VMAT2 and the implications a disturbance in the control by environmental agents has on Parkinson's disease and neuropsychiatric disorders. Intricate neuroanatomical analyses on brain tissue from Parkinson's patients shows that the highest levels of DAT and VMAT2 are found in the regions of the brain most sensitive to damage in Parkinson's disease. Studies using reserpine, a powerful inhibitor of VMAT2, suggest that disruption of vesicular loading of dopamine may play a role in depression. Control of DAT and VMAT2 has been implicated in other diseases such as Tourette syndrome, schizophrenia, drug and alcohol abuse, and attention deficit hyperactivity disorder.

Implications: These studies suggest that the control of DAT and VMAT2 plays a major role in mediating susceptibility to neurodegenerative and other diseases. It is possible that drugs designed to alter their balance might be beneficial in Parkinson's disease and other disorders. For patients in early stages of Parkinson's disease, reducing DAT activity might not only increase the synaptic dopamine concentration but might also slow the progression of the disease if toxins are present. In the case of VMAT2, drugs or therapies that increased vesicular uptake by VMAT2 decrease cytosolic dopamine perhaps by preventing its oxidation and storage of more usable dopamine. In conclusion, recent studies indicate that DAT and VMAT2 mediate neuronal damage and may influence susceptibility to a variety of neurological and psychiatric disorders.

Miller GW, Gainetdinov RR, Levey AI, Caron MG: Dopamine transporters and neuronal injury. Trends in Pharmacological Science, 20(10):424-429. 2000.

The Fidelity of DNA Synthesis by Human DNA Polymerase ?, A Skin Cancer Susceptibility Gene Product

Background: Humans and other organisms have the ability to respond to and repair DNA damage resulting from chemical and physical insults from the environment. Studies in recent years have revealed that the mechanisms for doing this are extremely complex and depend on the interplay between dozens of different proteins. Key among these proteins are the many DNA polymerases that copy DNA. Most DNA polymerases have great difficulty copying damaged DNA, yet we know this does occur in cells. Last year, two independent groups of scientists in the US and Japan discovered a novel human DNA polymerase which they designated DNA polymerase ? (Greek letter eta). This polymerase has the unprecedented ability to efficiently copy DNA containing the most common type of damage caused by exposure of skin cells to sunlight. These scientists also found that mutations that inactivate DNA polymerase ? are one cause of *Xeroderma pigmentosum*. This hereditary disease syndrome is characterized by a 1000-fold increase in susceptibility to sunlight-induced skin cancer.

Advance: In collaboration with the Japanese research group, NIH scientists have identified an unusual property of DNA polymerase ? that likely explains its ability to easily copy sunlight-induced DNA damage. DNA polymerase ? is extraordinarily inaccurate, making more mistakes when copying DNA than any other polymerase ever studied. This strongly indicates that DNA polymerase ? does not depend on the strict molecular shape identification tags that other DNA polymerases use to make new DNA. This less demanding requirement for precise geometry may allow it to efficiently bypass bulky DNA damage. The NIH scientists also discovered other properties that ideally suit this polymerase for its important role in damage bypass.

Implications: The types of DNA damage resulting from exposure to sunlight are only a few of the many damages that can arise from environmental exposures, both chemical and physical. Many of these DNA lesions are potentially highly mutagenic and the resulting mutations may contribute to a number of human diseases and to aging. As the first of what may soon be several known human polymerases to copy these various types of DNA damage, DNA polymerase ? is the paradigm for investigating the mechanisms and consequences of lesion bypass or its failure. Such studies are expected to improve our understanding of how mutations in DNA polymerase genes or their partners may increase the incidence of human diseases.

Matsuda T, Bebeneck K, Masutani C, Hanaoka F, Kunkel TA: Low fidelity DNA synthesis by human DNA polymerase ?. Nature, 404:1011-13, 2000.

Hidden Conformational Epitopes of HIV Envelope Protein Identified

Background: Acquired Immune Deficiency Syndrome (AIDS) has become the latest scourge of humankind. Caused by a family of viruses called Human Immunodeficiency Viruses, or HIV, this disease destroys the immune system and has been the focus of extensive research for nearly two decades. Much of this work has concentrated on understanding individual components of HIV, including HIV envelope glycoprotein, HIV-gp120, which plays a critical role in HIV infection by binding to receptors on the virus's target cells. This glycoprotein is also the primary target of antibodies that are trying to neutralize HIV. These antibodies bind to specific sites on the target molecule, called epitopes. Discovery of the particular epitopes that are recognized by antibodies specific for different parts of the HIV-gp120 molecule can be an important part of the development of HIV vaccines.

Advance: NIH scientists, using mass spectrometric techniques applied to this problem for the first time, have identified the 3-dimensional shape of epitopes (i.e., proteins eliciting an antigenic or immune response) area on the C5 region of the HIV envelope protein HIV-gp120 which are recognized by a human monoclonal antibody, 1331-A, isolated from a human HIV-infected patient. This antibody has high binding affinity for primary HIV isolates as well as laboratory-adapted strains. It has been observed that the core epitope of an antibody may only contribute 10% of the binding energy. This study revealed the conformational nature of the epitope – the fact that its recognition by the antibody depended on its being "folded" properly – and showed which parts of the protein other than the core epitope were involved in recognition of the protein by the antibody.

Implications: Knowledge of the epitopes of HIV-gp120 is crucial for an understanding of what parts of HIV could lend themselves to attack by the body's defenses. The information provided by mass spectrometric characterization of the tertiary structure of the C5 region of HIV-gp120 may be useful for vaccine development.

Hochleitner EO, Gorny MK, Zolla-Pazner S, Tomer KB: Mass spectrometric characterization of a discontinuous epitope of the HIV envelope protein HIV-gp120 recognized by the human monoclonal antibody 1331A. Journal of Immunology, 164(8):4156-61. 2000.

New Study Increases Understanding of Imprinting

Background: Most of the thousands of genes a person inherits come in two copies, and both function the same way. However, in the case of some genes, such as those that control the production of certain growth factors, one of the two copies of a gene is switched "off," while the other is switched "on." This process is called "imprinting." Scientists have suggested that imprinting protects organisms that would otherwise be harmed if both copies of the gene were actively producing proteins in a "double dose." For example, overexpression of certain genes in humans – such as the gene for insulin-like growth factor 2 (Igf2) – is associated with fetal overgrowth and, in some cases, the development of tumors. When imprinting occurs, the expressed gene may derive from either the mother or the father. Whether the maternal or paternal gene is "silenced" in imprinting depends on the presence of markers, called methyl groups, that chemically modify the DNA near the gene. In the case of insulin-like growth factor 2, the copy inherited from the father is functional and has methylated DNA nearby, whereas the copy from the mother is unmethylated and inactive. In cases in which the maternal copy of the gene is inappropriately methylated, there is overexpression of the protein, which is associated with a disorder that predisposes children to certain malignancies. A cutting-edge research area includes studies to determine what factors play a role in gene expression. Certain regions of DNA are known to contain genetic material that promotes or enhances gene activity, while others have inhibitory effects.

Advance: For insulin-like growth factor 2, scientists have discovered insulators of gene expression (the process by which a gene's coded information is activated). Investigators report that a certain protein, CTCF, acts to prevent the gene from making the growth factor. The team had been studying this protein because it binds to a "boundary" element between the loosely configured DNA where genes are active and the more tightly packed DNA where genes are inactive. Previous work had shown that this protein binds to specific sites on DNA and insulates, or blocks, gene-activating elements called "enhancers" from the genes they help potentiate. Based on these data, the group hypothesized that this protein played a similar role in ensuring that the maternal copy of the Igf2 gene remained switched off. Further studies showed that DNA near the Igf2 genes of mice and humans did indeed contain binding sites for this protein. Using cells in culture, researchers next showed that these binding sites can only block the action of an "enhancer" on the gene when the binding sites lie between the enhancer and gene, a signature of insulator activity. In the case of the Igf2 gene, the protein binding sites are found in the same region of DNA that is usually methylated on the paternal copy of the gene and unmethylated on the maternal copy. In the final experiment, the team showed that adding methyl groups to the DNA binding sites for this protein prevents further binding of the protein. This finding strongly suggests that methylation abolishes the insulating capability of the protein on the paternal copy of the gene. As a result, the enhancer can activate paternal gene expression.

Implications: Scientists have long recognized the importance of methylation in imprinting, but many of the other mechanisms governing imprinting remained unknown. A leading investigator in the field had hypothesized earlier that the Igf2 region contained an insulator that prevented the maternally inherited

gene from expressing growth factor and that methylation blocked this insulator. This research confirms the hypothesis. More importantly, the research demonstrates that the control mechanism for insulating against the activity of this imprinted gene is a novel "boundary" element, which the investigators are calling the "imprinting control region." Also, the fact that protein binding, and therefore insulator activity, can be turned "on" and "off" by changing the methylation state of DNA suggests that there may be many other places in the genome where a similar mechanism suppresses expression of a gene. The research team plans to continue its studies of the structure and function of insulators and related boundary elements to determine if this is actually the case. A practical implication of this new knowledge of how insulators regulate gene expression is its application to the design of "vectors" used in gene therapy in order to overcome problems with low gene expression associated with conventional vector design.

Bell AC, Felsenfeld G: Methylation of a CTCF-dependent boundary controls imprinted expression of the *IGF2* gene. Nature, 405(6785):482-5, 2000.

Saitoh N, Bell AC, Recillas-Targa F, West AG, Simpson M, Pikaart M, Felsenfeld G: Structural and functional conservation at the boundaries of the chicken ß-globin domain. European Molecular Biology Organization, 19(10):2315-22. 2000.

Recillas-Targa F, Bell AC, Felsenfeld G: Positional enhancer-blocking activity of the chicken ß-globin insulator in transiently transfected cells. Proceedings of the National Academy of Sciences, 96(25):14354-59. 1999.

Bell AC, West AG, Felsenfeld G: The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell, 98(3):387-96. 1999.

Explaining the Death of Insulin-Secreting Pancreatic Cells

Background: Type 2 diabetes is characterized by inadequate levels of insulin production, as well as a resistance to the action of insulin. Insulin is a hormone produced by the beta cells of the pancreas that helps the body use the sugar glucose for energy. The beta cell is responsible for controlling blood glucose levels. To accomplish this, the beta cell must "sense" the level of glucose in the blood and secrete the appropriate amount of insulin when glucose rises, to direct the uptake and storage of glucose. The number of beta cells can increase in response to the body's need for insulin, and then can also decrease by a feedback mechanism of programmed cell death, or "glucose-induced apoptosis." In genetically susceptible individuals, excessive glucose-induced apoptosis may decrease insulin production to the point where type 2 diabetes develops.

Glucose is also used in the modification of proteins through a process called glycosylation. Here, glucose and glucosamine are covalently linked to certain amino acids forming a "O-GlcNAc-modified protein." Interestingly, the enzyme required for O-GlcNAc protein modification has been found in high concentrations in the beta cell. Other data shows that a beta cell toxin, streptozotocin (STZ), long used to induce diabetes in experimental animals, blocks the enzymatic removal of the O-GlcNAc modification from proteins. This now appears to contribute to the programmed death of these cells. This data suggests a link between the utilization of glucose for protein modification and the ability of the beta cell to adapt to changes in blood glucose levels.

Advance: Research conducted and supported by the NIH has now provided direct evidence for a link between the pathway of glucose metabolism that leads to O-GlcNAc-modified proteins and glucose-induced apoptosis. First, NIH-supported researchers demonstrated that the amount of O-GlcNAc modified protein in the beta cell increased markedly in response to hyperglycemia in an animal model. After administration of insulin and the subsequent return to normal glucose levels, the level of O-GlcNAc modified protein returned to that seen in control animals. Next, the researchers established that STZ was toxic to the beta cell because it blocked removal of the O-GlcNAc protein modification. Lastly, using a mouse model with impaired glucosamine metabolism, researchers demonstrated that the action of STZ to block repair of O-GlcNAc modified proteins is the mechanism by which it causes beta cell death. Concomitantly, researchers at the NIH cloned and characterized the human enzyme involved in O-GlcNAc protein modification. The researchers then examined the ability of the enzyme to modify proteins involved in the regulation of glycogen synthesis (sugar storage). They found that it was able to modify two enzymes critical to this process. Taken together, these data suggest that the protein-modification function of glucose is of vital importance for beta cell adaptation to changes in glucose levels.

Implications: This is the first demonstration of a potential mechanism underlying the effect of severe or prolonged hyperglycemia in producing beta cell death and subsequent type 2 diabetes in genetically susceptible animals. Most importantly, this research has identified a potential target for prevention of

type 2 diabetes. Investigators are now working to solve the three dimensional structure of the enzyme involved in O-GlcNAc protein modification, an important step toward rational drug development targeted at interfering with this mechanism of beta cell death.

Lubas WA, Hanover JA: Functional expression of O-linked GlcNAc transferase: Domain structure and substrate specificity. Journal of Biological Chemistry, 275(15):10983-8. 2000.

Roos MD, Hanover JA: Structure of O-linked GlcNAc transferase: mediator of glycan-dependent signaling. Biochemical and Biophysical Research Communications, 271(2):275-80. 2000.

Liu K, Paterson AJ, Chin E, Kudlow JE: Glucose stimulates protein modification by O-linked GlcNAc in pancreatic ß cells: Linkage of O-linked GlcNAc to ß cell death. Proceedings of the National Academy of Sciences, 97(6):2820-5. 2000.

Bell AC, West AG, Felsenfeld G: The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell, 98(3):387-96. 1999.

Controlling Appetite Through Triglyceride Metabolism

Background: Obesity increases an individual's risk for developing cardiovascular disease, osteoarthritis, some forms of cancer, and type 2 diabetes. Scientists have now identified several hormones that are produced in fat cells that target the brain's hypothalamus to regulate body weight, either by stimulating or suppressing appetite. Two recent studies have explored the pathways of triglyceride metabolism as a means of controlling obesity.

Advance: Triglycerides are composed of glycerol and fatty acids and provide the body with a source of stored energy for metabolic functions. The last step in the synthesis of triglycerides is mediated by an enzyme called acyl CoA:diacylglycerol acyltransferase (Dgat). Because triglycerides play such an important role in metabolic functions, this enzyme was initially believed to be required for viability. Researchers have now bred a strain of genetically engineered mice that no longer has the gene that codes for this enzyme. Surprisingly, they grow and develop normally and are even capable of synthesizing triglycerides, suggesting that there are alternative pathways for triglyceride synthesis. Importantly, these mice are lean, and are resistant to diet-induced obesity. The leanness is the result of an increased metabolic rate and higher levels of physical activity, which result in increased energy expenditure without a compensatory increase in appetite and food intake. These mice have appropriately low serum levels of leptin, an important hormone in weight regulation, demonstrating that Dgat does not act through a leptin metabolic pathway. Investigators also tested the effect of treating mice with two fatty acid synthetase (FAS) inhibitors, cerulenin and the synthetic compound, C75. Results demonstrated a profound dose-dependent weight loss with the administration of either cerulenin or C75. Additional studies with C75 revealed that food consumption was significantly reduced over the first 24 hours after treatment, then returned to normal during the following 24 to 48 hours. These mice lost both lean body as well as adipose mass, which is the same pattern of weight loss in fasting. However, treated mice lost 40 percent more weight than equally-fed mice, indicating that loss of Dgat activity caused loss of appetite without the usual compensatory decrease in energy expenditure. C75 inhibits neuropeptide Y (NPY), a protein that regulates feeding status and adiposity, providing evidence that decreased appetite, at least in part, results from blocking NPY-induced feeding. Researchers found that C75-mediated FAS inhibition results in increased levels of the FAS substrate, malonyl-CoA, and that an inhibitor of malonyl-CoA restores appetite. These results support the theory that malonyl-CoA mediates the metabolic signal for appetite inhibition.

Implications: Knockout mice lacking the enzyme Dgat provide an important tool for investigating triglyceride synthesis and its relationship to obesity. Studies suggest that molecules that target triglyceride synthesis may prove to be exciting new approaches to treatment. Drugs that mimic the actions of C75 by inhibiting FAS synthetase may cause appetite inhibition while sustaining an increase in metabolic rate, and would possibly provide ideal agents for treatment of obesity.

Smith SJ, Cases S, Jensen DR, et al: Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nature Genetics, 25(1): 87-90. 2000.

Loftus TM, Jaworsky DE, Frehywot GL, et al: Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science, 288(5475):2379-8. 2000.

New Insights Gained into Genetics and Treatment of Polycystic Kidney Disease

Background: Polycystic kidney disease (PKD) affects about 500,000 people in the United States, and is the fourth leading cause of kidney failure. A disease of genetic origin, it is characterized by massive enlargement of the kidneys associated with growth of multiple fluid-filled cysts. Scientists have discovered mutations in the genes known as *PKD1* and *PKD2* that are responsible for the development of autosomal dominant PKD (ADPKD). Subsequently, researchers discovered a family of proteins produced by the PKD genes, called polycystins.

Advances: Scientists have made several advances in understanding the most prevalent form of PKD since discovery of the causative genes. Using three mouse models, scientists found that, depending on the severity of the mutation, the animals either died before birth of major kidney, heart, and pancreas defects, or had decreased length of survival. They concluded that the presence of polycystin-2 is essential for normal development of parts of the kidney, heart, and pancreas. A second research team examined kidney cysts from two patients and discovered that 71% of the cysts had mutations in the *PKD2* gene, while a subset of cysts lacked that mutation but had mutations in the *PKD1* gene. The findings suggest that *PKD1* mutations may be modifiers of disease severity, and that independent disturbances in the production of the polycystin proteins by the PKD genes may be sufficiently disruptive to cause cyst formation. In another series of experiments, researchers built on insights in cell signaling mechanisms to show that a newly-developed inhibitor of epidermal growth factor receptor tyrosine kinase activity (EKI-785) reduced cysts, improved kidney function, decreased liver abnormalities, and increased life span in a mouse model. The drug acts on an enzyme critical for growth factor signaling. When drug treatment was stopped, the disease returned.

Implications: Beyond the current understanding of how to identify and treat complications of PKD, the breakthrough discovery of PKD genes opened new avenues of research on the most prevalent form of the disease. These include the determination of the proteins produced by the genes and how they function in the normal and disease states, and the interactions of the abnormal genes that lead to disease. These research avenues will advance understanding of the molecular and cellular events in PKD so that safe and effective therapies can be developed. In addition, further research on growth factor signaling pathways could form the basis for starting human trials leading to an effective treatment for PKD.

Wu G, Markowitz GS, Li L, et al: Cardiac defects and renal failure in mice with targeted mutations in *Pkd2*. Nature Genetics, 24(1):75-8. 2000.

Watnick T, He N, Wang K, et al: Mutations of PKD1 in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. Nature Genetics, 25(2): 143-4. 2000.

Sweeney WE Jr, Chen Y, Nakanishi K, Frost P, Avner ED: Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor. Kidney International, 57(1):33-40. 2000.

Hematopoietic Stem Cells Pave Way to New Treatments for Disease

Background: Stem cells hold great promise for advances in medical care because they can give rise to many different types of specialized cells that may be used to replace or repair damaged tissues and organs and for other therapeutic purposes. One type of stem cells is called "hematopoietic," because it normally gives rise to the spectrum of adult blood cells. These cells originate in the bone marrow and in the developing liver. Little is known about the events that cause these cells to commit to specific cell lineages or about their genetic program, that is, the specific genes they express and proteins the cells produce. The use of many new techniques in genomics and bioinformatics is enabling scientists to dissect out and understand relationships between genes involved in regulatory processes in stem cells and ultimately will clarify the genetic basis for cell differentiation.

Advance: A major discovery is the primitive myeloid progenitor cell in the mouse. First, researchers separated all of the hematopoietic and lymphoid progenitors from bone marrow cells using specific markers for those cell types. From the remaining cells, sub-populations were separated using other specific cell markers. The commitments to lineages of megakaryocytes/ erythrocytes (platelet and red blood cell progenitors) and macrophages/granulocytes (white blood cell progenitors) were shown to be mutually exclusive events, with these two sub-populations derived from a common myeloid progenitor. No lymphoid cells were produced from any of the sub-populations. A second group of researchers reported using a combination of analytic techniques – high-throughput DNA sequence acquisition, bioinformatic and array analyses, and new hybridization techniques – to elucidate novel stem cell genes that may give insights into how these cells develop further into more mature cell types. From cells extracted from fetal mouse liver tissue, scientists identified at least 161 transcription factors, which are involved in ultimate direction of protein synthesis; 174 cell surface molecules; 28 secreted proteins; and 147 signaling molecules. Many of these were previously undescribed or were found in stem cells for the first time. Comparison with genes of stem cells derived from other sources revealed several genes that may have regulatory functions in hematopoietic stem cells.

Implications: These studies set the stage for future investigations of genes involved in the commitment of cells to specific lineages, and also for investigations of the transformation events leading to the multitude of blood disorders. Use of molecular analytic techniques will permit study of changes in the genetic program, i.e., protein expression, depending on whether the stem cell is renewing itself or is maturing into a more committed and developed cell type. The discovery of stem cell proteins will facilitate study of networks of protein interactions, known as proteomics. This knowledge will be critical for future research that can produce specialized cells for treating disease.

Akashi K, Traver D, Miyamoto T, Weissman IL: A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature, 404(6774):193-7. 2000.

Phillips RL, Ernst RE, Brunk B, et al: The genetic program of hematopoietic stem cells. Science, 288(5471):1635-40. 2000.

Insights into the Role of the Immune System in the Development of Type 1 Diabetes

Background: The immune system consists of a variety of cells whose coordinated actions protect the body from bacteria, viruses, and other harmful agents. Detection of foreign antigens – proteins that do not occur naturally within the body – alerts the immune system to the presence of a threat. One critical aspect of the immune system is its ability to differentiate between self and foreign and to attack only those cells that exhibit foreign antigens. If the immune system loses this ability, the body may attack itself. In people with type 1 diabetes, the individual's immune system destroys the insulin-producing cells of the pancreas, requiring regular injections of insulin for survival.

Advance: One of the first signs of an immune response is the production of antibodies, proteins that assist the body in the removal of foreign agents. Patients with type 1 diabetes often develop antibodies against proteins in the insulin-producing pancreatic beta cells, including insulin itself. A recent study examined whether the presence of insulin autoantibodies (IAA) could identify individuals likely to develop type 1 diabetes. Blood samples were taken from over 900 infants at about nine months of age and IAA levels measured. By one year of age, five individuals with elevated levels of IAA were found; four of them subsequently developed type 1 diabetes before their fourth birthdays (the fifth is only 2 years old). In contrast, only one infant, of the remaining 900 or so infants whose IAA levels were not initially elevated, developed an elevated level of IAA after one year of age and also developed disease. Thus, an elevated IAA in one-year-old infants predicts subsequent onset of type 1 diabetes by their fourth birthday.

In order to understand exactly how the cells of the immune system distinguish between normal and potentially harmful proteins, researchers have examined the Major Histocompatibility Complex of proteins (MHC). MHC is present on the surface of immune cells and helps identify potentially harmful antigens. Scientists found that mice susceptible to diabetes had an MHC that was shaped differently from normal ones. This minor change in shape produces an MHC that interacts with a wider range of antigens, and may be prone to misidentify normal occurring benign proteins as threatening. Some people with type 1 diabetes have a MHC whose structure is similar to that of the diabetic mice, suggesting that a similar mechanism may lead to development of the disease in both.

Implications: The finding that high IAA levels correlate with later development of diabetes may help to identify at-risk populations and allow prevention before overt symptoms develop. Insights into how abnormal MHC proteins identify autoantigens as threatening may provide new opportunities for preventive therapies. It may be possible to design drugs that specifically inhibit the interaction with these autoantigens, while still permitting the immune system to identify potentially harmful antigens. Collectively, these studies may enable identification of individuals at risk for the development of type 1 diabetes and allow for intervention prior to irreversible damage to the insulin producing cells in the pancreas.

Yu L, Robles DT, Abiru N, et al: Early expression of antiinsulin autoantibodies of humans and the NOD mouse: Evidence for early determination of subsequent diabetes. Proceedings of the National Academy of Sciences, 97:1701-6. 2000.

Corper AL, Stratmann T, Apostolopoulos V,et al: A structural framework for deciphering the link between I-Ag7 and autoimmune diabetes. Science, 288:505-11. 2000.

New Understanding of Insulin Receptors and Insulin Resistance in Type 2 Diabetes

Background: People with type 2 diabetes may continue to produce insulin but their bodies do not respond normally to the action of insulin, a phenomenon termed "insulin resistance." In order to respond to insulin cells must have insulin receptor (IR) proteins on their surface. Binding of insulin to its receptor triggers a cascade of events within the cell that results in the uptake of glucose from the blood. In order to characterize the proteins that relay the insulin signal within the cell, scientists studied engineered mice lacking several proteins important in the insulin signaling pathway: the IR itself and two other proteins that mediate insulin action, IRS-1 and IRS-2. Because each gene is normally present in two copies, or "alleles," mice can be produced that lack one or both alleles. The ability of the cells and tissues of these animals to respond to changes in glucose levels can then be compared to that of normal mice with two functional alleles.

Advance: Researchers have examined the roles of IR, IRS-1, and IRS-2 in regulating the response of different tissues to changes in blood glucose levels. Mutant mice were generated with combined mutations which inactivated one allele of the insulin receptor (*ir*) gene, and an allele of one of the insulin receptor substrate genes (*irs*-1) or (*irs*-2). Despite similar rates of diabetes in these two lines of mice *(ir/irs-1*$^{+/-}$ and *ir/irs-2*$^{+/-}$*)*, closer examination reveals variations in the tissues affected. Mice with a mutation in the *irs*-2 gene exhibit severe insulin resistance in the liver but only mild defects in muscle. In contrast, mice with a mutation in the *irs*-1 gene exhibit severe insulin resistance in both muscle and liver. Pancreatic beta cells respond to increases in blood glucose levels by producing and releasing insulin. To gain insight into the role of insulin signaling in this process, scientists isolated islets from mice lacking both IRS-1 alleles. These cells contained significantly less insulin than normal cells. Furthermore, the absence of IRS-1 resulted in significant defects in insulin secretion, as evidenced by the relative insensitivity of the beta cells to changes in glucose levels.

Implications: These studies emphasize that the responsiveness of a given tissue to changes in glucose levels is complex and requires the coordination of multiple proteins. Mice lacking various combinations of IR and IRS-1 or IRS-2 exhibit different profiles of insulin resistance in various tissues despite having similar overall rates of diabetes. This result indicates that, while IRS-1 and IRS-2 are closely related molecules, each protein performs unique functions and that one factor cannot fully compensate for the absence of the other. This uniqueness of function seen in the IRSs lends support for the view that type 2 diabetes is a polygenic inherited disease. The finding that beta cells that are insulin resistant have an impaired ability to produce and secrete insulin suggests that the development of insulin resistance in these cells may reduce the amount of insulin available to other tissues of the body. Such a development could exacerbate the problems in regulating blood glucose levels. Ways to address this problem will be the subject of much future research.

Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, Accili D: Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. The Journal of Clinical Investigation, 105:199-205. 2000.

Kulkarni RN, Winnay JN, Daniels M, Bruning JC, Flier SN, Hanahan D, Kahn CR: Altered function of insulin receptor substrate-1-deficient mouse islets and cultured ß-cell lines. The Journal of Clinical Investigation, 104(12):69-75. 1999.

Insights into the Molecular Mediators of Hyperglycemic Damage to Blood Vessels

Background: In diabetes, the mechanism by which elevated blood glucose leads to long-term complications is incompletely understood. Recently, it has become clear that hyperglycemia may cause damage to the endothelial cells, which line blood vessels, leading to micro- and macrovascular abnormalities. This damage can be caused, in part, by a chemical reaction between glucose and proteins on the endothelial cell surface. Cells whose proteins have been glycosylated no longer function normally and may become targets for immune system attack. A fuller understanding of the glucose-protein interaction and cellular responses might permit development of therapies to limit injury to the blood vessel and surrounding tissue.

Advance: Scientists have recently found that a protein called CD59 – bound on the surface of endothelial cells – is a target for modification by glucose. CD59 functions normally to inhibit the activation of complement, a component of the immune system, during an inflammatory response. The activation of complement triggers a cascade of events, including release of growth factors, which cause abnormal proliferation of cells in the vessel wall. In experimental models, glucose-modified CD59 no longer inhibits complement activation, leading to proliferative changes in the vessels, characteristic of the abnormalities seen in diabetic complications. Additional insights into the molecular pathways of glucose-mediated damage within the cell have come from studies of bovine arterial cells in culture. Hyperglycemia is known to be associated with high levels of an unusual form of oxygen, known as superoxide. Superoxide is extremely unstable and can damage a large number of cellular components. Investigators have recently demonstrated that mitochondria are an important source of superoxide in diabetes. Furthermore, normalizing mitochondrial superoxide prevented the activation of four different biochemical pathways, each of which has been implicated in the pathogenesis of diabetic complications. Previously, it was not clear how these seemingly separate pathways might be related in the development of hyperglycemia-induced cellular damage. Inhibition of multiple intracellular pathways that are activated in response to elevated glucose levels, by inhibiting the production of superoxide, suggests a common end point for several pathways of cellular damage.

Implications: To date, no single treatment aimed at inhibiting a single cellular pathway implicated in the development of diabetic vascular damage has proved successful in preventing or ameliorating complications. Identifying superoxide production as a potential final common pathway for multiple mechanisms activated by high glucose levels may lead to more effective strategies to inhibit damage to blood vessels. Identification of CD59 as a target for modification by glucose also represents an important step forward in understanding of diabetes. Modified CD59 can be detected in patients, and agents that block this reaction may inhibit the development of vascular complications. Interestingly, the region of CD59 modified by glucose is present only in the human form of the protein, offering a possible molecular explanation for some of the uniquely human aspects of the disease and possibly explaining why this disease has proven so difficult to replicate completely in animal models.

Nishikawa T, Edelstein D, Du XL, et al: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature, 404(6779):787-90. 2000.

Acosta J, Hettinga J, Fluckiger R, et al: Molecular basis for a link between complement and the vascular complications of diabetes. Proceedings of the National Academy of Sciences, 97:5450-5. 2000.

Research on Nuclear Receptors May Aid Drug Development

Background: The actions of steroids and related hormones on their target cells are mediated through binding of the hormone to specialized proteins within the cell called nuclear hormone receptors (NRs). Some NRs are already present in the nucleus, bound to specific regions of DNA. Others are present in the cell cytoplasm. Hormone binds to the NR and either helps it to get to the nucleus or, once there, to interact with a series of nuclear accessory proteins. The end result is to turn on or off specific genes. Some of the accessory proteins, known as repressors and activators, stabilize their inactive and active forms, respectively. Understanding these processes is important because a number of diseases, including several forms of cancer, arise from inappropriate or unregulated signaling by NRs.

Advance: The creation of new drugs to inhibit NR activation often requires detailed information about the structure of the NR. Knowledge of the NR in the absence of the antagonist is of limited use because NRs often assume an altered shape when bound to inhibitors. Researchers have created a computer program that extrapolates from the structure of one well-characterized NR-inhibitor complex to perform virtual experiments with potential new drugs. Using this approach, scientists screened over 150,000 chemical compounds and identified two novel potential antagonists for an NR. This strategy was validated when subsequent laboratory experiments confirmed that these factors inhibited activity of this NR. Further insight into the function of NRs comes from studies of activators and repressors. Scientists have studied two repressors that associate with NRs in the absence of hormone and stabilize their inactive state in order to identify which regions of the proteins are important for their function. Researchers found that the interaction between these repressors and their target NRs was mediated through a relatively small region within the repressors – a CoRNR box. This CoRNR box is surprisingly similar to the NR box in activators that mediates binding to activated NRs. The importance of NR and CoRNR boxes in discriminating between active and inactive NRs was shown in experiments using proteins engineered to possess various combinations of these regions. A hybrid activator engineered to contain CoRNR boxes instead of NR boxes acted as a repressor, indicating that a very small area of the activator may be responsible for its activity.

Implications: The evolution of new therapies may be greatly accelerated by the use of computers to perform virtual surveys of hundreds of thousands of drugs. This can only work when researchers have elucidated the structures of hormone receptors, such as the Nuclear Receptors. By allowing a much larger pool of potential therapeutic agents to be surveyed, novel or previously unexplored agents may be identified. The identification of specific regions in NR repressors that mediate binding to nuclear receptors pinpoints sequences that may be important in the future development of therapeutic agents. Agents that target the CoRNR box and inhibit binding to the NR promote nuclear receptor activation, and may pave the way to designing agents for use in humans.

Hu X, Lazar MA: The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. Nature, 402(6757):93-6. 1999.

Schapira M, Raaka BM, Samuels HH, Abagyan R: Rational discovery of novel nuclear hormone receptor antagonists. Proceedings of the National Academy of Sciences, 97:1008-13. 2000.

Genomes of Yeast, Worm, and Fly Aid Understanding of Human Disease

Background: From single-celled organisms to multicelled animals, many of the basic biochemical activities important to life are remarkably similar. Once scientists have uncovered the functions of genes or proteins in one organism, they have clues to the functions of related genes or proteins in other organisms, including humans. Since researchers have now sequenced the genomes of three major organisms – the fruit fly (*Drosophila melanogaster*), the worm (*Caenorhabhitis elegans*), and the yeast (*Saccharomyces cervevisiae*) – there are vast opportunities for interspecies comparisons of genes and proteins. Because these three model organisms have been so thoroughly studied over the years, the functions of many of their proteins and genes are either already known or can be determined through further experimentation.

Advance: A multicenter team has started to analyze comparative data on the genomic and protein sequences of the fruit fly, worm, and yeast. Specifically, the researchers determined how many distinct protein families each genome encodes, how the genes encoding these protein families are distributed in each genome, and how many genes are shared among flies, worms, yeast, and mammals. The scientists then compiled a dataset of nearly 300 human genes known to contribute to a variety of human diseases, and they searched for similar genes in the fly, worm, and yeast. The fly was found to have the most corresponding genes, with DNA sequences that resembled more than 60 percent of the human disease genes in the dataset.

Implications: The study uncovered several previously unrecognized fruit fly counterparts to human disease genes that contribute to colon cancer, muscular dystrophy, Parkinson's disease, and other disorders. These genes can now be closely examined in the fly to provide new insights into the human diseases. Overall, inter-species comparisons of fundamental genetic and cellular processes promise to enhance our understanding of the basic underpinnings of human biology. This, in turn, may offer clues to new, highly targeted strategies for treating or preventing a variety of human diseases.

Rubin GM, Yandell MD, Wortman JR, et al: Comparative genomics of the eukaryotes. Science, 287(5461):2204-15. 2000.

The Feasibility of Large-Scale Mutagenesis and Phenotyping Programs

Background: The past few years have seen rapid advances in the deciphering of the genomes of humans, mice, and other animal species. The next major challenge will be to assign functions to the approximately 100,000 genes in each of these genomes. Laboratory mice are the most commonly used species to investigate gene function, in part because scientists are able to produce engineered mutations, or variants, through a variety of evolving technologies. Although large collections of mice with specific mutations have been discovered or engineered over the past 100 years, these mutations represent only several thousand of the approximately 100,000 genes in the mouse genome. In many cases, the effects of these mutations have not yet been completely clarified. There is thus a significant shortfall in knowledge about the function of most of the genetic material.

Advance: In an extensive overview of this subject, researchers have assessed the current state of mouse genomics and phenotyping (assigning functions to genes). The assessment describes the original work with spontaneous mutants, gene-based studies utilizing DNA analysis, and large-scale mutagenesis projects using chemicals to induce changes in the genome. The latter method produces extensive numbers of mutant animals, which need to be assessed to determine the effects of the mutations. This leads to the need for significant increases in the level of phenotyping activity, which is proving to be the rate-limiting step in large-scale studies. The scientists propose that novel genetic resources – such as extensive phenotype databases and standardized assays for determining phenotype – will be needed to help speed the discovery of gene and protein functions in the mouse.

Implications: Although scientists are producing an increasing number of genetically altered mice, efforts to correlate genotype with phenotype are lagging behind. A concerted effort is needed to standardize the study of phenotype, develop rapid techniques for identifying the subtle effects of genes, and characterize interactions between genes in the mouse. Just as improved technologies allowed scientists to scale up genome analysis for the Human Genome Project, novel techniques must be explored for scaling up analysis of phenotype in the mouse and other organisms. With expanded information on mouse phenotypes, scientists could identify mouse models of human disease more efficiently.

Nadeau, JH: Muta-genetics or muta-genomics: the feasibility of large-scale mutagenesis and phenotyping programs. Mammalian Genome, 11(7):603-7. 2000.

A Genetic Linkage Map of the Baboon Genome

Background: Nonhuman primates are valuable animal models for the study of human diseases. Their close evolutionary relationship to humans presents opportunities for comparative analyses of genome structure and gene function, known as genomics. In many cases, however, scientists have not yet determined which genes are located on each of the chromosomes in nonhuman primates. This placement of specific genes or gene functions at particular sites in the genome is known as genetic linkage mapping. The development of genetic linkage maps for nonhuman primates would provide new opportunities for diverse lines of research and for comparative studies in human genetics and gene function.

Advance: Core staff scientists at the Southwest Regional Primate Research Center and their collaborators have developed a genetic linkage map for the baboon. The mapping results confirm and extend many previously published conclusions regarding comparative chromosome structure between humans and nonhuman primate species. Direct comparison of equivalent areas of the two primate genomes showed that for seven non-sex-related human chromosomes, genes are located in the same order in humans and baboons. For the other 15 non-sex-related chromosomes, one or more rearrangements distinguish the two genomes. The baboon linkage map is the first reported for any nonhuman primate species. The similarities to the human linkage map open new opportunities for studying genes in the nonhuman primate that may be involved in diseases of humans.

Implications: Development of complete gene linkage maps for various primate species will create new research opportunities in biomedical and evolutionary genetics, including genome scanning for genes and comparative genomics. Nonhuman primates already play a critical role in biomedical research. Expanding this role to include modern genomic and genetic linkage analyses will significantly increase the range of studies possible, as well as provide new insights into the human genome itself.

Rogers J, Mahaney MC, Witte SM, et al: A genetic linkage map of the baboon (*Papio hamadryas*) genome based on human microsatellite polymorphisms. Genomics, 67(3):237-47. 2000.

MRI Reveals Changes in Brain Structure Associated with Multiple Sclerosis

Background: Multiple sclerosis (MS), an inflammatory disease of the central nervous system, occurs in two different forms: relapsing-remitting and progressive. In many patients, the progressive form of the disease occurs after a relapsing-remitting stage, but in some patients the disease is progressive from onset. Because of these different forms of the disease, and because of limitations in the traditional clinical scales used to assess the disease, there is a need for an accurate and efficient measurement of disease status.

Advance: Researchers at Brigham and Women's Hospital used magnetic resonance imaging (MRI) to observe lesions in patients with different forms of MS. Each patient had up to 24 MRI scans, scheduled over a 12-month period. Sophisticated analysis of these images showed differences between patients with relapsing-remitting MS and those with progressive MS. The MRI images clearly correlated with the traditional clinical scales used to evaluate the progression of multiple sclerosis.

Implications: This work demonstrates that MRI can be used to evaluate clinical disability in the study of multiple sclerosis. Such a finding is important because it indicates that MRI can serve as an objective measure of patient response to experimental treatments. In addition, these results further define the features seen in MRI that are associated with the different stages of MS.

Weiner HL, Guttmann CRG, Khoury SJ, Orav EJ, Hohol MJ, Kikinis R, Jolesz FA: Serial magnetic resonance imaging in multiple sclerosis: Correlation with attacks, disability, and disease stage. Journal of Neuroimmunology, 104(2):164-73. 2000.

Application of Laboratory Research to the Development of a Therapy for Chronic Myelogenous Leukemia

Background: Leukemias are cancers that affect white blood cells. Chronic myelogenous leukemia (CML), sometimes called chronic granulocytic leukemia, affects white blood cells called granulocytes as they are formed in the bone marrow. CML prevents granulocytes from maturing properly. The abnormal cells become too numerous and cannot do their job of protecting the body against infection. Most cases of CML can be detected by identifying a distinctive chromosome that appears in nearly all patients with this disease. For some time it has been known that this chromosome, named the Philadelphia (Ph) chromosome, is unique to tumor cells and that nearly all patients with CML carry the genetic mutation that gives rise to it. It has also been known that the Ph chromosome causes the production of an enzyme known as a tyrosine kinase. However, the underlying mechanism by which the disease arises and progresses remained a mystery until studies showed that the presence of the Bcr-Abl protein alone is enough to cause CML. Further studies established that the tyrosine kinase activity produced by this protein is required for the disease to progress. These findings led to investigations of whether a drug could be developed specifically to inhibit the production of this tyrosine kinase, thereby slowing or halting the progress of the disease.

Advance: The drug, designated STI571, inhibits tyrosine kinase activity. In both cell cultures and living animals, STI571 was found to either stop the growth of abnormal granulocytes or cause them to self-destruct. In those studies, investigators found no abnormal effects on normal cells. Conducting studies in human patients, the researchers further found that all patients with chronic-phase CML who were treated with STI571 had a remission of their disease. Several patients even showed a complete disappearance of the Ph chromosome. Researchers have yet to find any toxic effects of the experimental drug.

Implications: These studies show that patients with CML who carry the Ph chromosome can provide a unique opportunity to study not only the onset of the disease, but also the use of treatments that suppress or inhibit the processes that lead to the progression of CML. This work is an important demonstration of how laboratory research can eventually be translated into the development of a powerful new therapeutic agent for the treatment of cancer.

Druker BJ, Lydon NB: Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia. The Journal of Clinical Investigation, 105(1):3-7. 2000.

Sawyers CL, Druker B: Tyrosine kinase inhibitors in chronic myeloid leukemia. Cancer Journal from Scientific American, 5(2):63-9. 1999.

Predicting Lung Cancer by Detecting Methylated Genes

Background: Lung cancer, already the leading cause of cancer death in the United States, is expected to reach epidemic proportions throughout the world during this century. Efforts to reduce mortality due to many types of cancer have included the development of molecular markers. These are unique signatures of cancer cells that can be used to detect cancer in its earliest stages, before it can be found by other means and when treatment is more likely to be successful. An ideal DNA marker should be detectable early enough in the disease process for treatment to improve the patient's prognosis. It should also be easy to detect in a fluid or tissue that can be collected from patients without the need for invasive procedures. Although significant advances have been made in the identification of such markers for many types of cancer, none has yet been found suitable for use in detecting early lung cancer. Because both current and former cigarette smokers have increased secretions, or sputum, from the bronchi – the branch-like passageways leading from the throat to the lungs – studies of DNA markers for lung cancer have focused on analyzing genes isolated from the sputum of former and current smokers with lung cancer. Recent studies by NIH-supported researchers may provide clues for developing DNA markers for early lung cancer.

Advance: The investigators selected two genes in particular for study. The *p16* gene plays a key role in regulating cell cycles, and *MGMT* protects cells from the cancerous effects of substances like cigarette smoke. Both genes are good candidates for molecular markers of early lung cancer. The researchers looked for very specific molecular changes in these genes, which were isolated from sputum samples of patients with a form of lung cancer known as squamous cell carcinoma (SCC). These patients included current and former smokers, as well as individuals exposed to radon through mining. Scientists were looking for *methylated* genes – those in which an extra chemical group was present. Methylation of the promoter region of these genes, which can occur through exposure to cigarette smoke, effectively inactivates the gene. When genes such as *p16* and *MGMT*, which normally help to protect against cancer, become methylated, cancer may be more likely to develop. Methylation of one of these genes was found in the sputum of every one of the patients with SCC studied by the researchers. This chemical change was seen not only at the time of diagnosis, but also in all the sputum samples that had been collected from 5 months to nearly 3 years *before* lung cancer could be clinically detected.

Implications: Detecting methylation in the promoter region of genes may hold great promise for finding lung cancer at a very early stage. It may also be possible to apply this method to the detection of other forms of cancer.

Palmisano WA, Divine KK, Saccomanno G, et al: Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Research, 60(21):5954-8. 2000.

Gene is a Critical Player in Tumor Metastasis

Background: The most damaging change that can occur during cancer progression is the spread of cancerous cells to organs distant from the primary site of disease. In this process, known as metastasis, the cells break off from tumors, enter the bloodstream, and travel to other organs, where they grow into new tumors. Metastasis is ultimately responsible for the deaths of most cancer patients, but it remains a poorly understood process. Researchers do know that, in order to metastasize, tumor cells must complete a complex series of steps that involve numerous molecular changes. A complete understanding of the molecular basis by which tumors spread to distant organs is critically important to the development of new strategies to diagnose, control, and treat metastatic cancer.

Advance: Using modern molecular technology that allows the analysis of several thousand genes at a time, NIH-funded investigators have identified a gene that causes noninvasive, poorly metastatic melanoma cells to become invasive and metastatic. The investigators created a panel of mouse and human melanoma cells, some of which were metastatic and some of which were not. By analyzing the genes expressed by each cell, they tried to identify genes involved in changing tumor cell behavior, causing the cells to become highly invasive and metastatic. This gene analysis produced several promising candidate genes, one of which – rhoC – is known to be involved in tumor cell motility and invasion. When rhoC was expressed in poorly metastatic human melanoma cells, the cells became highly motile and metastatic. In contrast, when rhoC expression in metastatic cells was inhibited, the cells became less motile and were poorly metastatic. The investigators are currently testing the hypothesis that rhoC can confer metastatic properties on other human tumor cells.

Implications: These results demonstrate that molecular characterization of tumor cells can provide more refined and clinically useful definitions of tumors by differentiating those that are malignant but noninvasive from those that are invasive and metastatic. Such studies also provide opportunities to develop more effective strategies for diagnosis and treatment.

Clark EA, Golub TR, Lander ES, Hynes RO: Genomic analysis of metastasis reveals an essential role for Rho C. Nature, 406(6795):532-5. 2000.

Anti-Apoptosis Gene is Overexpressed in Cancer Cells

Background: Apoptosis, or programmed cell death, is a normal physiological process that eliminates unneeded or damaged cells and prevents the overgrowth of cells. It plays an essential role in embryonic development as well as in the daily maintenance of body systems. Disruption of normal apoptosis contributes to many diseases, including cancer. For example, if a mutation occurs in a gene that induces apoptosis, cells may fail to respond to the cue to die. Instead, the cells proliferate uncontrollably, forming a cancerous tumor. Apoptosis is currently the focus of intense interest by cancer researchers who hope that a better understanding of this process will help to explain how cancer arises and point the way to the development of new treatment strategies.

Advance: NIH-supported investigators have discovered a gene that appears to be a critical regulator of apoptosis and to be particularly important in cancer. This gene, named survivin, is the smallest member of a family of genes known as inhibitors of apoptosis. The investigators have demonstrated that survivin is abundantly expressed in many malignant tumors, including basal and squamous cell skin cancers, metastatic melanoma, and bladder cancer. However, survivin is not expressed in normal tissue adjacent to the tumors. The researchers have also shown in model tumor cell lines that blocking survivin expression results in spontaneous apoptosis. A recent genomic analysis found that survivin was invariably expressed in cancer but not in normal tissues. Other data suggest that survivin plays a key role in cell development by preventing apoptosis during cell division. When survivin is overexpressed, as it is in cancer cells, its anti-apoptosis function may allow cells that should have been destroyed to proliferate. Another line of investigation has shown that survivin is highly expressed in the newly formed blood vessels of tissue that forms over a healing wound. The growth of new blood vessels, or angiogenesis, is essential for tumor growth.

Implications: These findings suggest that survivin holds promise both as a marker of cancer progression and as a possible target for therapeutic intervention. Since survivin appears to have an anti-apoptosis function, it is possible that blocking its expression could promote apoptosis in cancer cells. Additionally, since survivin seems to play a key role in angiogenesis, blocking its expression could inhibit the blood-vessel development that is essential for tumor growth. These approaches will need to be tested in animal models and, if they continue to show promise, in clinical trials.

O'Connor DS, Schechner JS, Adida C, et al: Control of apoptosis during angiogenesis by survivin expression in endothelial cells. The American Journal of Pathology, 156:393-8. 2000.

Grossman D, McNiff JM, Li F, Altieri DC: Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. Journal of Investigative Dermatology, 113(6):1076-81. 1999.

Li F, Ackermann EJ, Bennett CF, et al: Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. Nature Cell Biology, 1(8):461-6. 1999.

Clue from Inherited Childhood Disorder May Help Explain Breast Cancer

Background: Ataxia telangiectasia is an inherited disorder that causes progressive ataxia (loss of movement coordination) beginning usually between ages 1 and 2. Blood vessel abnormalities called telangiectases on the eyes and skin account for the name of the disease. In addition to loss of certain brain cells, children with this disease often suffer immune deficiency, increased likelihood of cancer, and abnormally high sensitivity to radiation. Each year about 500 people inherit "bad" copies of the relevant gene from both parents, and thus the disease ataxia telangiectasia. About 1% of the population – more than 2 million people – carry one bad gene. Carriers are spared most problems of this disorder but may have four times increased risk of cancer and increased sensitivity to radiation.

About 5 years ago scientists, supported by NIH and others, discovered the ATM gene which, when defective, causes ataxia telangiectasia. Subsequent study, by these investigators and others, revealed that the normal ATM gene helps prevent a cell from becoming cancerous when its DNA is damaged.

Advance: NIH-supported scientists have now discovered a link between the cellular functions of the ATM gene and the BRCA1 gene for inherited breast cancer. The normal BRCA1 gene plays a critical role in cells' repair response to DNA damage. The normal function of the ATM gene is critical in a step that alerts the BRCA1 gene that DNA damage has occurred. This may explain the increased risk for cancer among children with ataxia telangiectasia and carriers of one bad ATM gene. More generally, these findings are helping to elucidate how cells normally repair DNA damage and how compromise of this repair system contributes to cancer.

Implications: These findings are an important step towards understanding both ataxia telangiectasia and cancer. Like many inherited diseases, discovery of the ATM "disease gene" has not led immediately to treatments, but progress is continuing, building step-by-step on that discovery. Because DNA repair mechanisms are critical for many neurological, immune, and cancer disorders, the continuing story of research also highlights how progress in one disease, sometimes rare, can be a key to understanding cells' signal and control pathways that are involved in many other diseases.

Li S, Ting NSY, Zheng L, Chen PL, Ziv Y, Shiloh Y, Lee EYHP, Lee WH: Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. Nature, 406(6792):210-15. 2000.

Chemokines in Multiple Sclerosis: Prospects for Better Drugs

Background: Multiple sclerosis (MS) is a chronic disease that usually strikes in the early adult years. In MS a person's own immune cells cause inflammation in the brain and spinal cord and destroy the myelin that ensheathes nerve fibers and is essential for reliable conduction of high speed nerve impulses. So, when myelin is disrupted, nerve impulses carrying sensory and movement signals deteriorate, and people with MS suffer a host of debilitating problems. Despite new therapies that can slow the progression of MS, treatment for the 250,000+ U.S. citizens with this disorder remains unsatisfactory.

Fundamental studies of the immune system have produced a wealth of information that is now being applied to MS. Researchers can distinguish which of the many types of immune cells are the culprits in MS. Biologists have also found perhaps 100 different chemical signals that control critical steps in immune activity. Progress in understanding about one of these, interferon, led to therapies that reduce MS attacks and slow the progress of the disorder, but better therapies are needed. Because the chemical signals of the immune system act on diverse aspects of immune function and different immune cell types respond differently, selective therapeutic strategies for inflammatory diseases should be feasible. The challenge is to discover which signals and cells are involved in each step of the MS disease process and to use that information to develop better treatments.

Advance: An international collaborative effort has now revealed specific immune control chemicals that are critical in MS. A series of studies implicates certain members of a family of chemical signals called chemokines that attract immune cells to tissue and stimulate inflammation. The levels of two chemokines (IP-10 and RANTES), but not others, are increased in the cerebrospinal fluid of patients during acute MS attacks, but not brain inflammation from other causes. Further studies of which chemokines affect immune cells that infiltrate the brain supported the notion that these two signals are critical in MS. Other investigations of human tissue and animal models of MS suggest that astrocytes, a type of non-neuronal cell in the brain, produce IP-10 during MS.

Implications: Chemokines and their receptors, the cells' detectors of these signals, are especially good targets for developing potent and specific drugs because there are many of them (more than 50); they act quite specifically on different types of immune cells; and chemokine receptors belong to a class of molecules called G-protein coupled receptors through which many drugs act. There is currently rapid development of small-molecule that block chemokine receptors, many of which are approaching clinical trials for varied conditions including HIV infection, transplant rejection, asthma and rheumatoid arthritis. The finding that specific receptors and chemokines are highly expressed in the cells and tissues of the brain in patients with MS provides a first step towards the rational use of these agents in clinical trials, although we still need to know much more about the precise functions of these chemokines and receptors in MS.

Sorensen TL, Tani M, Jensen J, et al: Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. The Journal of Clinical Investigation, 103(6):807-15. 1999.

Mouse Model of Neurofibromatosis Developed

Background: Although not well known by the public, neurofibromatosis is among the most common genetic diseases that affect the nervous system. It is more prevalent than Tay-Sach's disease, cystic fibrosis, and Huntington's disease combined. In the most common form of this disorder, neurofibromatosis type 1 (NF1), patients usually develop multiple tumors (neurofibromas) along nerves. Individuals with NF1 can have a range of other problems, including learning disabilities, pigmentary abnormalities, bone deformities, and other benign and malignant tumors. Approximately a decade ago, the genetic defect that causes NF1 was found, providing hope that a cure will one day be discovered. A mouse model of NF1 would provide a powerful tool in understanding the disease and developing therapies. However, previous studies showed that defects in the mouse *NF1* gene are not sufficient to cause neurofibromas or the malignant tumors that can lead to death in NF1 patients.

Advance: Two teams of scientists recently developed mouse models of NF1. These laboratories showed that inactivating two genes, the mouse version of the *NF1* gene and a second gene called *p53*, causes mice to develop malignant tumors resembling those that can occur in NF1 patients. One of the groups also used a specific genetic methodology to show that mice with a defective *NF1* gene can be made to develop the benign neurofibromas so common in human patients.

Implications: These mouse models of NF1 will be invaluable for two reasons. First, they will allow investigators to understand how this often devastating disease develops. For example, scientists still do not know what kind of nerve cell is affected by defects in the *NF1* gene. Identifying the cell type that gives rise to NF1-associated tumors will be critical for understanding the disorder. Second, these mice will allow investigators to quickly test drugs that may block tumor development. Such studies may provide a basis for subsequent clinical trials in humans.

Vogel KS, Klesse LJ, Velasco-Miguel S, Meryers K, Rushing EJ, Parada LF: Mouse tumor model for neurofibromatosis type 1. Science, 286(5447):2176-9. 1999.

Understanding the Early Steps in Neurodegeneration

Background: Spinocerebellar ataxia-1 (SCA1) is an inherited disorder characterized by loss of movement coordination (ataxia), usually beginning in adulthood, reflecting the progressive death of nerve cells in the cerebellum, the spinal cord, and other parts of the brain. In 1993 scientists discovered that defects in a previously unknown gene, called ataxin-1, cause SCA1. SCA1 is one of eight known neurological disorders, including Huntington's disease, that are caused by "triplet repeat expansions," with each disease involving a different gene. In the genetic code a triplet designates an amino acid, one of the building blocks of proteins. In these diseases the expansions consist of many abnormal repetitions a triplet. For all of these diseases the triplet for the amino acid glutamine is repeated, so cells make proteins with long stretches of glutamines inserted. Scientists have genetically engineered SCA1 mice that mimic the human disease, and they are trying to unravel how the "polyglutamine" containing proteins lead to neurodegeneration.

Advance: By studying gene expression scientists have found important clues about the earliest steps in neurodegeneration in SCA1. Genes carry the blueprints for proteins, and proteins are the workhorses of cells, so studying gene expression, that is, which genes are turned on in particular cells, provides essential clues to normal and disease physiology. Scientists compared gene expression in normal and SCA1 mice using a technique called "PCR-based cDNA subtraction" which copies and amplifies active gene messages and highlights the differences between diseased and normal animals. Six genes, all abundant in the cerebellum, are downregulated at a surprisingly early stage of the disease. Some of these genes produce proteins that are important in regulating calcium within cells. Cells use calcium to control many critical internal processes, such as the release of neurotransmitters, and abnormal calcium levels have been previously linked to neurodegeneration. The mouse version of a human gene called alpha-ACT-1 is also perturbed in SCA1 mice. This too suggests links to other disease because ACT-1 is affected in Alzheimer's and Huntington's diseases.

Implications: In most neurodegenerative disorders nerve cells begin to sicken and die long before the first signs of disease become apparent. Because the brain has limited capacity to repair itself, the best hope is to understand the earliest steps in neurodegeneration and stop the process. Although SCA1 and the other triple repeat disorders are far less common than diseases like Alzheimer's and Parkinson's, the well defined genetic causes present advantages for study of the early processes of neurodegeneration. Accumulating evidence increasingly implicates processes like protein aggregation, calcium dysregulation, and cell suicide in many common and uncommon neurodegenerative disorders. These basic findings in SCA1 will not lead to immediate clinical applications, but may ultimately lead to treatments that interfere with early steps in neurodegeneration.

The study is also an important prototype in another sense. Tools from the Human Genome Project are allowing scientists to rapidly identify the mutated genes that cause hundreds of neurological disorders. Moving from discovery of a previously unknown gene to understanding of disease processes is not easy. This study illustrates how studies of gene expression can help.

Xi L, Antalffy B, Kang D, Orr HT, Zoghbi HY: Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. Nature Neuroscience, 3(2):157-63. 2000.

How Nicotine Causes Long Lasting Effects on the Brain

Background: The effects of nicotine on the brain are primarily responsible for why smoking is addictive. Scientists have long known that nicotine mimics acetylcholine, a natural neurotransmitter in the brain, by strongly activating one class of nerve cells' detectors for this molecule, the "nicotinic" acetylcholine receptors. Earlier experiments have also shown that acetylcholine helps control the activity of the "reward" centers in the ventral tegmental area (VTA) of the brain. What has been difficult to explain is how even a brief exposure to nicotine can have very long lasting effects on the brain.

Advance: Now scientists, working with slices of the rat VTA in a dish, have shown that nicotine acts through a mechanism called long-term potentiation that has been linked to learning and memory. Nicotine acts on the nerve terminals that release the neurotransmitter glutamate. This induces long-term potentiation which strengthens synapses onto cells that release the transmitter dopamine. Dopamine plays a critical role in addictive behaviors.

Implications: This finding reveals a potential target for medications that could help smokers quit. Because this nicotine effect appears to act through only one subtype of receptor (the alpha 7 subunit), such future drugs might be quite specific in their actions.

Mansvelder HD, McGehee DS: Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron, 27(2):349-57. 2000.

The Normal Development of the Olfactory System is Dependent on Neuronal Activity

Background: One of the fundamental challenges in developmental neurobiology is to determine the role stimulus-induced or spontaneous neuronal activity plays in the normal development of sensory systems. For example, it is well known that pattern recognition and binocularity are dependent on retinal activity during a critical developmental period in the visual system. In the olfactory system, NIH-funded investigators have shown that the loss of sensory input due to nares blockage, olfactory epithelium damage, or frontal head trauma causes severe but reversible biochemical and structural changes in the olfactory bulb, which are accompanied by altered odor perception. Functional recovery occurs after odorant accessibility is re-established or following regeneration of the injured olfactory epithelium and/or nerves. Since olfactory receptor cells are actually specialized neurons that project directly to the olfactory bulb, an understanding of the cellular and molecular biological mechanisms underlying such activity-dependent plasticity may help to address the broader neurobiological question about factors that regulate the recovery of function in other damaged areas of the central nervous system.

Advance: A recent NIH-funded study examined the importance of odor-induced neuronal activity in establishing the projection patterns of individual olfactory receptor neurons, and in establishing normal olfactory function. In the olfactory system, a single odorant receptor is expressed in each olfactory receptor neuron. Ordinarily, the olfactory receptors neurons that express the same odorant receptor send converging projections to 1-2 glomeruli of the olfactory bulb. In order to test the hypothesis that such projections are activity-dependent, NIH-supported scientists used genetic techniques to eliminate a key molecule in the excitation of olfactory neurons that respond to a certain type of odorant. In the knockout mutation, the odor-receptor and G-protein interactions are intact but the olfactory receptor neuron is non-excitable. These investigators found that in the olfactory receptor neurons that expressed the P2 odorant receptor, the projections to the olfactory bulb were altered. These findings were interpreted to indicate that odorant-induced neural activity shaped and pruned the projections of olfactory receptor neurons, and the lack of such activity results in aberrant projections that presumably affect olfactory coding.

Implications: This is the first study of the olfactory system that identifies the importance of olfactory neuron activity in determining appropriate connections with the olfactory bulb.

Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P: Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. Neuron, 26(1):81-91. 2000.

Rapid Progress in the Mapping and Cloning of Genes Responsible for Hereditary Hearing Impairment

Background: A number of NIH-supported investigators have made rapid progress in mapping and cloning the genes responsible for hereditary hearing impairment.

Advance: In the past few years, the chromosomal locations of over 60 genes whose mutations result in hereditary hearing impairment have been determined. In the past two years, the genes responsible for 14 common hearing disorders have been cloned and the specific genetic mutation leading to the disorder has been identified.

Implications: This highly successful effort has several fundamental implications for treating individuals with genetically based hearing impairment. Identification of the genes, or DNA sequences, that mediate hearing loss enables the scientist or clinician to rapidly identify individuals carrying a defective gene even if the hearing loss has a delayed onset and is not yet evident. Furthermore, each mutation in a specific deafness gene is correlated with specific physiological characteristics. The opportunity arises for improved medical treatment and monitoring based on the clinical profile known to be associated with a specific DNA mutation.

Identification and isolation of genes responsible for hereditary hearing impairment provides a powerful means to study how the mutation results in deafness. Many successful genetic approaches include mutation or deletion of the identified gene in an animal model such as the mouse. Study of the genetically modified animal can provide critical information including which structures of the ear are affected and the specific molecular and physiological defects that result in hearing impairment. This information is potentially valuable for identifying possible ways to inhibit or treat progressive deafness. In addition, creation of an animal model of the disease also provides a system in which to test potential new therapies.

McGuirt WT, Prasad SD, Griffith, AJ, et al: Mutations in COL11A2 cause non-syndromic hearing loss (DFNA13). Nature Genetics, 23(4):413-19. 1999.

Bykhovskaya Y, Estivill X, Taylor K, et al: Candidate locus for a nuclear modifier gene for maternally inherited deafness. American Journal of Human Genetics, 66(6):1905-10. 2000.

Kumar S, Deffenbacher K, Marres HAM, et al: Genomewide search and genetic localization of a second gene associated with autosomal dominant branchio-oto-renal syndrome: clinical and genetic implications. American Journal of Human Genetics, 66(5):1715-20. 2000.

Salam AA, Hafner FM, Linder TE, et al: A novel locus (DFNA23) for prelingual autosomal dominant nonsyndromic hearing loss maps to 14q21-q22 in a swiss german kindred. American Journal of Human Genetics, 66(6):1984-88. 2000.

Hafner FM, Salam AA, Linder TE, et al: A novel locus (DFNA24) for prelingual nonprogressive autosomal dominant nonsyndromic hearing loss maps to 4q35-qter in a large swiss german kindred. American Journal of Human Genetics, 66(4):1437-42. 2000.

Morell RJ, Frederici KH, Wei S, et al: A new locus for late-onset, progressive, hereditary hearing loss DFNA20 maps to 17q25. Genomics, 63(1):1-6. 2000.

Kelley PM, Abe S, Askew JW, et al: Human connexin 30 (GJB6), a candidate gene for nonsyndromic hearing loss: molecular cloning, tissue-specific expression, and assignment to chromosome 13q12. Genomics, 62(2):172-6. 1999.

The Molecular Biology of Taste Signal Transduction: Diversity Personified

Background: A long history of NIH-funded behavioral, electrophysiological, and biochemical research has shown that taste perception involves the four basic taste qualities of sweet, sour, salty and bitter. More recent molecular biological data suggest that "umami" taste (a.k.a., monosodium glutamate) or the taste associated with protein-rich foods may be a fifth basic taste quality. Each taste quality appears to be mediated by a distinct signal transduction pathway. Salty (Na^{++} ions) and sour (acidic H^{+} ions) activate specialized ion channels in the cell membrane of the taste receptor cell; sweet-, bitter-, and umami-tasting substances activate G-protein-coupled receptors.

Advance: A series of elegant molecular biological studies have characterized the diverse structure, function, and expression of a novel family of 40–80 G-protein coupled receptors, the so-called T2Rs, which are selectively expressed in taste receptor cells of the tongue and palate epithelium. T2R receptors map to gene loci that have been reported to influence bitter taste perception in humans and mice, suggesting that T2R are indeed bitter receptors. T2R receptors are also linked via the G-protein gustducin to the internal signal transduction machinery of taste receptor cells. Gustducin is critical for the transduction of responses to bitter-tasting and possibly sweet-tasting compounds. This evidence supports the interpretation that T2Rs are functional bitter receptors. Importantly, in a functional expression assay, T2Rs have been shown to couple to gustducin *in vitro* and to respond to a bitter tastant.

Several NIH-funded laboratories are exploring the variety of signal transduction mechanisms associated with bitter and sweet taste receptors. Research suggests that different bitter and/or sweet receptors may activate distinct second messenger cascades, and that different bitter compounds (such as caffeine) may activate different signal transduction circuits, providing a basis for bitter perception. These studies have made significant contributions to the understanding of the structural and functional properties of taste receptors associated with sweet- and bitter-taste quality perception. Similar approaches are being developed to explore the uniquely modified G-protein coupled, metabotropic glutamate receptor that is likely to be involved in umami perception.

Implications: These genetic and molecular biological studies have made significant contributions to understanding the structural and functional properties of taste receptors associated with sweet- and bitter-taste quality perception. Similar approaches are being used to study a glutamate receptor variant that is likely to be involved in umami, or monosodium glutamate (MSG), perception.

Huang L, Shanker YG, Dubauskaite J, et al: G?13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium. Nature Neuroscience, 2(12):1055-62. 1999.

Chaudhari N, Landin AM, Roper SD: A metabotropic glutamate receptor variant functions as a taste receptor. Nature Neuroscience, 3(2):113-19. 2000.

Adler E, Hoon MA, Mueller KL, et al: A novel family of mammalian taste receptors. Cell, 100(6):693-702. 2000.

Chandrashekar J, Mueller KL, Hoon MA, et al: T2Rs function as bitter taste receptors. Cell, 100(6):703-11. 2000.

Obese Mouse Reveals New Approach to Building Bone

Background: In normal bone, cells called osteoclasts dissolve, or resorb, bone. Bone resorption is a normal part of bone remodeling, in which old or damaged bone is replaced with new bone. New bone is formed by cells called osteoblasts. Net loss of bone, leading to osteoporosis, occurs when bone resorption exceeds bone formation. Such an imbalance can occur either because osteoclast numbers or activity increases, or because osteoblast numbers or activity decreases. The most common cause of bone loss is the decline in the female sex hormone estrogen in women after menopause. However, bone loss can also occur because of the effects of certain steroid drugs or when mechanical loading of the skeleton is reduced, as in prolonged inactivity or spaceflight.

Advance: Studying genetically obese strains of mice, investigators noticed that the mice were defective in the action of a chemical called leptin. Leptin acts through the central nervous system to control food intake along with several other aspects of behavior and physiology. In the absence of leptin's normal action, the mice become very fat. Usually, high body weight results in high bone mass. However, these mice also have defects in the development of sex organs, hypogonadism, and have high levels of a naturally occurring chemical called cortisol, hypercortisolism, which has effects similar to steroid drugs. Because hormones produced by sex organs are necessary to maintain bone mass, and steroids like cortisol usually cause bone loss, the researchers examined the bones of the mice.

Surprisingly, in spite of the hypogonadism and hypercortisolism, the mice had very high bone mass. Further, the high bone mass was not due to obesity, but instead to the absence of leptin function. It seems that leptin normally acts to suppress the bone-forming activity of osteoblasts. Leptin is thought to act mainly through a part of the central nervous system called the hypothalamus. This discovery reveals a previously unknown mechanism by which bone formation is regulated.

Implications: Because little is known about the biochemical mechanisms that control the activity of osteoblasts, it has proven difficult to design therapies (anabolic) that would induce net formation of new bone. The leptin-deficient mice are remarkable in that high bone mass coexists with hypogonadism and hypercortisolism, two conditions that normally lead to bone loss. This suggests that leptin, acting through the hypothalamus, may actually mediate the deleterious effects of sex hormone deficiency and steroid excess. If drugs can be designed to block leptin's action, they may be useful as anabolic therapies.

Ducy P, Amling M, Takeda S, et al: Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. Cell, 100(2):197-207. 2000.

Mice Reveal a New Target for Prevention of Bone Loss

Background: For several years, researchers have studied special proteins called proton pumps because it seemed likely that protein pumps might perform an osteoclast-specific function. (Osteoclasts break down, or resorb, bone – a normal part of the remodeling process.) Proton pumps transport protons (hydrogen ions) across cell membranes. They are found in many different kinds of cells because the concentration of protons determines whether an environment is acidic, alkaline, or neutral. In most parts of the body, the environment is maintained close to neutrality. However, to dissolve the mineral components of bone, osteoclasts must pump protons into the tiny space between the osteoclast membrane and the bone surface, making this microenvironment highly acidic.

Advance: Investigators tested the biological function of a particular protein, *Atp6i*, believed to be part of a proton pump and present only in osteoclasts. They created a genetically modified mouse strain in which the *Atp6i* gene was inactivated. The mice exhibited severe skeletal abnormalities that are characteristic of a disorder called osteopetrosis, which is due to a deficiency of bone resorption during early growth. In addition, isolated osteoclasts from the modified strain were unable to resorb bone when tested in cell culture. Thus, it appears that the *Atpi6* protein is essential for bone resorption.

Implications: This work suggests that a drug designed to block *Atp6i* function could be a specific inhibitor of bone resorption, and thus might provide a new approach to preventing bone loss in a variety of situations. Because *Atp6i* seems to be osteoclast-specific, it may be possible to block its action without deleterious effects on other tissues, an important factor in pharmacological therapy.

Li YP, Chen W, Liang Y, et al: *Atp6i*-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nature Genetics, 23(4):447-51. 1999.

Correlation Found Between Genetic Defect and Appearance of Skin Disease

Background: The ichythyoses are a group of hereditary and acquired diseases that result in abnormally thickened skin or skin structures. A number of genetic abnormalities in the main building block of the stratum corneum, the keratins, have been isolated over the years. The bank of specific abnormalities is becoming large enough to make correlations between the genetic defect and the appearance of the disease.

Advance: Epidermolytic hyperkeratosis is a fairly severe form of ichthyosis. It has been shown to result from defects in either keratin 1 or keratin 10. In recent research, a distinct clinical form of epidermolytic hyperkeratosis was found wherein the disease manifested in psoriasis-like plaques. Genetic mutation analysis revealed a unique mutation in the keratin 1 gene. This is a specific example of the value of continued genetic analysis to identify specific genotype-phenotype correlations.

Implications: Genetic studies of keratins in the various forms of ichthyosis will provide an understanding of the basis of disease and could make possible earlier intervention and prediction of disease severity.

Michael EJ, Schneiderman P, Grossman ME, Christiano AM: Epidermolytic hyperkeratosis with polycyclic psoriasiform plaques resulting from a mutation in the keratin 1 gene. Expimental Dermatology, 8(6):501-3. 1999.

Both Embryonic and Aging-Related Genes May Regulate the Aggressive Behavior of Joint Lining Cells in Rheumatoid Arthritis

Background: Rheumatoid arthritis (RA) is a chronic inflammatory condition that affects large and small joints and is characterized by inflammation of the joint lining membrane (synovium), cell proliferation, development of new blood vessels, and destruction of the underlying cartilage and bone.

Advance: Recent studies suggest that expression of genes that regulate cell fate may play a role in synovial inflammation in RA. In one study, investigators discovered that RA synovial cells express embryonic growth factors that, when cultured in the laboratory, produce inflammatory cytokines (substances involved in cell-to-cell communication). In another study, investigators observed a high expression of aging-related genes in RA tissues, which can result in cell proliferation and inflammation.

Implications: These findings suggest that intrinsic properties of synovial cells independent of the immune response may contribute to the local changes observed in joints of RA patients. This offers an opportunity to develop new therapies designed to modify the activities of synovial cells.

Sen M, Lauterbach K, El-Gabalawy GS, et al: Expression and function of wingless and frizzled homologs in rheumatoid arthritis. Proceedings of the National Academy of Sciences, 97(6):2791-6. 2000.

Two Related Proteins Form a Physical Complex with Calcineurin to Regulate Gene Expression of Muscle Fiber Types

Background: Adult skeletal muscle fibers can be divided into several subtypes based on speed of shortening, susceptibility to fatigue, and metabolic properties. Different fiber types respond differently to disease processes as well. Research has shown that the molecular basis for fiber type diversity is the existence of multiple forms of muscle contractile and metabolic proteins and that calcineurin is involved in muscle fiber specification.

Advance: Two related proteins that are expressed most abundantly in striated muscles have been found to form a physical complex with calcineurin. Abbreviated MCIP1 and MCIP2, these proteins appear to inhibit calcineurin activity, but their expression is markedly increased in muscles with slow fiber types as compared to faster fibers. This suggests that the interaction between MCIPs and calcineurin is complex and regulated by additional factors.

Implications: Muscle fiber type has a subtle but profound effect on human health. Slow fiber types and fast fiber types are associated with different risks for, and resistances to, various diseases, such as atherosclerosis and diabetes. Understanding the molecular pathway controlling muscle fiber characteristics makes possible development of interventions to enhance muscle performance and reduce risk of life-threatening diseases.

Rothermel B, Vega RB, Yang J, et al: A protein encoded within the Down syndrome critical region is enriched in striated muscles and inhibits calcineurin signaling. The Journal of Biological Chemistry, 275(12):8719-25. 2000.

Genes Express Differences in Tendon and Ligament Repair

Background: Injuries to the knee are common in sports activities and have been reported to be the most frequently encountered injuries seen by orthopaedic surgeons. The resulting instability following injury can result in functional disability and lead to the development of degenerative osteoarthritis. Injuries to the knee's extra-articular ligaments (MCLs) generally heal spontaneously, where injuries to the knee's intra-articular ligaments (ACLs) do not. Research has shown that primary cells from the ACL proliferate and migrate more slowly than those from the MCL.

Advance: To test the hypothesis that this difference in proliferation potential is the result of differences in the expression of specific genes in these ligaments, researchers used a recently developed technique (polymerase chain reaction-based subtractive cDNA analysis) to identify genes that are differentially expressed in MCLs and ACLs. They found differences exist in New Zealand white rabbits, expressed by different gene mutations in the tissues, at the gene level.

Implications: This finding opens a new vista to the development of techniques and reagents to study the differences between periarticular tissues that differ in their ability to self-repair.

Goomer RS, Maris T, Ostrander R, Amiel D: PT-12, a putative Ras-activated proliferation-dependent gene, is expressed in patellar tendon and not in anterior cruciate ligament. Journal of Orthopaedic Research, 17(5):745-7. 2000.

Research Yields Clues About the Cycling of Hair

Background: Hair is an appendage present in most mammals. It grows in cycles under the control of a number of genes and influenced by a number of proteins. An understanding of the events in hair development, cycling, and the mechanism of hair loss in various diseases will allow for the development of treatment to correct abnormalities.

Advance: Animal models are being used widely in the study of hair, particularly mouse models. In one investigation, a common protein (TGF-beta) implicated in a several diseases was found to play a role in hair follicle development and to induce the beginning of hair follicle formation. In another, involving hairless mice whose absence of hair is caused by a specific gene defect, it was shown that their hair regressed after the first cycle. Other studies have shown that when a specific protein (mouse keratin 6a) is genetically manipulated to be abnormal, severe hair loss is observed, and that a widely used treatment for the human disease of alopecia areata also works in animal models.

Implications: The rapidly expanding knowledge of how the hair follicle forms in cycles and how it is perturbed in disease is providing a basic understanding that will be relevant to other less accessible cycling cellular systems, such as cancer, as well as to treating hair diseases.

Shapiro J, Sundberg JP, Bisonnette R, et al: Alopecia areata-like hair loss in C3H/HeJ mice and DEBR rats can be reversed using topical diphencyprone. Journal of Investigative Dermatology Symposium Proceedings, 4(3):239. 1999.

McElwee KJ, Boggess D, Miller J, King LE Jr, Sundberg JP: Spontaneous alopecia areata-like hair loss in one congenic and seven inbred laboratory mouse strains. Journal of Investigative Dermatology Symposium Proceedings, 4(3):202-6. 1999.

A Neuroendocrine Model Explains Gender Differences in Behavioral Responses to Stress

Background: The "fight-or-flight" response is generally regarded as the prototypic human response to stress. Physiologically, it is characterized by sympathetic nervous system activation which ultimately results in the secretion of chemicals into the bloodstream mobilizing the behavioral response. Whether the response culminates in "fight" or "flight" is thought to depend on whether the threat or stressor is perceived as surmountable. Thus, an appropriate stress response is essential to survival. While this biobehavioral "fight-or-flight" theory has dominated stress research for the past 5 decades, it has been disproportionately based on studies of males. This is because females' greater cyclical variation in neuroendocrine responses presented a confusing and often uninterpretable pattern of results. As a result, the processes involved in stress responses in females are less well understood.

Advance: A team of NIH-supported scientists has formulated a theory that characterizes female responses to stress by a pattern they term "tend-and-befriend," rather than by "fight-or-flight." They believe that female stress responses have selectively evolved to simultaneously maximize the survival of self and offspring. Thus, the "tend-and-befriend" pattern involves females' nurturance of offspring under stressful circumstances, the exhibition of behaviors that protect them from harm (tending), and befriending – namely, creating and joining social groups for the exchange of resources and to provide protection. They propose that these responses build on the biobehavioral attachment-caregiving processes that depend in part on oxytocin, estrogen and endogenous opioid mechanisms for the down-regulation of sympathetic and hypothalamic-pituitary-adrenocortical (HPA) responses to stress. A substantial neuroendocrine literature from animal studies (rats and nonhuman primates) provides support for these proposed mechanisms. These neuroendocrine models link to humans in that oxytocin, coupled with endogenous opioid mechanisms and other sex-linked hormones, fosters similar maternal and affiliative behaviors in both animals and humans in response to stress. Finally, literature on both human and nonhuman primates evidence substantial female preference to affiliate under stress compared to males. The "tend-and-befriend" pattern likely is maintained not only by sex-linked, neuroendocrine responses to stress, but by social and cultural roles as well.

Implications: This interesting, new, theoretical model opens a fresh field of inquiry in stress research that has potential for closing some empirical gaps and addressing gender biases. For example, it examines other neurohormones (e.g., serotonin, prolactin, dopamine, etc.) that also may be implicated in these processes of stress-regulation, but that are not yet well understood in either males or females. It also examines the role of oxytocin in social bonds outside of the basic mother-offspring attachment processes commonly studied.

Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA: Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychological Review, 107(3):411-29. 2000.

Building a Brain Synapse: Understanding the Axonal "HOV" Lane

Background: A critical task for the developing brain is to establish the points of contact, called synapses, that enable neurons, or brain cells, to communicate. Neurons consist of a cell body and two tentacles – an axon and a dendrite – that extend out of the cell body to transmit and receive information. During brain development, proteins and other cellular components needed to construct a synapse move out of the cell body along the axon. Scientists have long thought that only after an axon terminal was positioned to contact an adjoining dendrite would these synapse-building components individually be transported in tiny bubble-like vesicles along the full length of the axon to the "construction site," much like a stream of traffic moving along a highway. Current advances challenge the established hypothesis.

Advance: NIH-sponsored researchers have found that the neuronal remodeling needed to build synapses occurs wherever dendrites or axons initiate cell-cell contact – that is, at many sites along an axon, and not just at the terminal. It appears that this on-site remodeling is possible because all the necessary components for a synapse are transported along a dendrite together in one vesicle, and not individually. Whereas transporting each component separately would require regrouping all necessary parts at the terminal before synapse construction could occur, these "high occupancy vesicles" make synapse construction possible at any number of sites. Scientists made this discovery by attaching a green fluorescent protein (GFP) to a common synaptic vesicle protein, called VAMP. The fluorescence permitted them to observe vesicle protein movement along the axon. They found that some fluorescently marked vesicle protein aggregates would remain stationary at the presynaptic terminal region of an axon. Other fluorescent protein aggregates traveled along the axon, toward the terminal, but occasionally stopped along the way and even moved backwards.

When cell-cell contact was initiated by dendritic extension, a fluorescently marked vesicle protein aggregate was recruited to the point of interaction, and the vesicle would discharge and recycle the materials needed to build a synapse. When growing axons came in contact with dendrites, the fluorescently marked vesicle protein complex was simultaneously localized at the point of contact. The investigators used protein-labeling techniques to determine that typical components of synaptic terminals, such as voltage-dependent calcium channels and synaptic vesicle proteins, are components of the vesicle protein aggregate; indeed, it appears that the fluorescently marked vesicle protein aggregate contains all of the components necessary for active restructuring and recycling at any of multiple points along axons and dendrites.

Implications: This study provides evidence for a new model of synaptic terminal formation. Understanding the plasticity demonstrated by axonal-dendritic interactions is not only important to development and regeneration of neurons, but could be particularly important in post-developmental synaptic remodeling – for example, the mechanisms involved in long-term memory. The work also promises to shed new light on many types of developmental neuropathology.

Ahmari SE, Buchanan J, Smith SJ: Assembly of presynaptic active zones from cytoplasmic transport packets. Nature Neuroscience, 3(5):445-51. 2000.

A Newly Discovered Protein Transports A Major Excitatory Brain Transmitter

Background: Mental and physiological health depend upon various chemical transmitter and receptor systems that make possible intercellular communication. Those systems depend, in turn, on the ability of proteins to properly package neurochemicals into synaptic vesicles, the launch-pads that release chemicals into extracellular space to communicate with adjoining neurons. *Excitatory* and *inhibitory* describe two major types of neurotransmitters that essentially turn the communications mechanisms of neurons "on" or "off." In order for neurotransmitters to be released from one cell and exert their actions on other cells, they have to gain access into a synaptic vesicle by means of a transporter. Research previously has shown that the major *inhibitory* transmitter, GABA, is pumped into its vesicle by the VGAT transporter. VGAT relies on both acidity and electrical charge to transport GABA. Transporters for other neurochemicals have been identified as well, but the transporter for glutamate, which is the major and most abundant *excitatory* neurotransmitter, has long eluded researchers – until now.

Advance: Years of experimentation led to the recent discovery and partial characterization of a glutamate transporter, called VGLUT1. In the course of working with a transporter that moves phosphate into synaptic vesicles, a group of NIH-sponsored investigators began to detect clues that this phosphate transporter also affected glutamate. Curious about the role of the transporter, they inserted it into a cell line that normally lacks it. They found that a preparation of synaptic-like vesicles containing the transporter were able to take up two to four times as much glutamate as preparations without the transporter. Closer examination revealed the transporter's specificity toward glutamate – for example, amino acids similar to glutamate did not gain access through the transporter. For many neurotransmitters, transport into a vesicle depends primarily on differences in local acidity. By contrast, uptake of glutamate through its transporter depends primarily on electrical charge and less on acidity.

Glutamate is not the only compound that uses the VGLUT1 transporter. Other molecules, including phosphate and chloride, also gain access to a vesicle through VGLUT1, and at this point, the relationship between glutamate and these other compounds is not clear. Also curious is why some brain regions appear to lack VGLUT1 when glutamate is essentially everywhere in the brain. In pursuit of answers to this question, scientists already have identified a different form of VGLUT, which could account for total brain glutamate-uptake.

Implications: Understanding the regulation and mechanics of brain glutamate-release is key in understanding many pathologies. Excess glutamate is thought to have a critical role in disorders ranging from alcohol withdrawal to stroke, to some psychiatric illnesses. Identification of the glutamate transporter clears a major hurdle in identifying key proteins involved in normal physiology and disease.

Bellocchio EE, Reimer RJ, Fremeau RT Jr, Edwards RH: Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. Science, 289(5481):957-60. 2000.

Single Neurons Play Complex Roles in Encoding Memories

Background: Within the past 15 years, a key advance in memory research was the discovery of a neural correlate of "working memory." This term refers to a very short-term form of recollection for events that have occurred in the immediate past – for example, your ability to remember a phone number long enough to dial it after looking it up in a telephone book. A simple task was devised to probe this form of memory in non-human primates. The task involves cuing monkeys to make eye movements to a particular location in space, but forcing them to wait (i.e., remember the cued location) for a brief period of time before making the eye movement. This work enabled researchers to discover specific neurons in a part of the prefrontal cortex that showed an enhanced rate of activity during the "memory" period prior to the eye movement. There quickly followed a series of interesting experiments investigating the role of this region of cortex in memory mechanisms. Scientists proposed several hypotheses concerning the structural and functional organization of this brain region. A prominent feature of these hypotheses concerned the organization of specific regions of prefrontal cortex into discrete areas responsible for processing distinct and different aspects of sensory information related to memory for events, i.e., what the stimulus was versus where it was located.

Advance: Recently, NIH-supported investigators challenged some of these hypotheses with a very clever series of experiments. The researchers trained monkeys to associate a high frequency tone with a red-colored light and a low frequency tone with a green-colored light. In the experiment after training, a monkey would hear a particular tone and would have to choose the appropriate order to receive a reward. Because the light choice was not offered until 10 seconds after the tone sounded, the animal had to "remember" which tone had occurred. The investigators found that single neurons in a specific region of prefrontal cortex (called dorsolateral prefrontal cortex) became active, showing a memory response during the delay period, and an enhanced response when the color choice was presented. Interestingly, specific neurons responded to the "high-frequency – red" combination while others responded to the "low frequency – green" combination. Even more fascinating were trials in which animals made an error (for example, choosing the red color when a low frequency tone had been delivered, i.e., an incorrect memory). In those instances, the firing pattern of neurons did not show the expected pattern of correlation between tone and color selection.

Implications: These findings show that the organization of this region of cortex is more complex than originally thought. Particular neurons are not tuned exclusively to a specific aspect of sensory information, but respond to multiple modalities (tone and color). Moreover, while the dorsolateral prefrontal cortex previously had been thought to be exclusively involved in coding the location ("where"), as opposed to the category ("what") of object, in these experiments these neurons were clearly coding object characteristics and not just their location.

Fuster JM, Bodner M, Kroger JK: Cross-modal and cross-temporal association in neurons of frontal cortex. Nature, 405(6784):347-51. 2000.

New Views On Brain Development

Background: Sophisticated brain imaging equipment, such as magnetic resonance imaging (1.5 Tesla MRI scanners) machines, are fairly new technologies that, because of cost and complexity, are found only in a few large research facilities. MRI technologies are powerful research tools that can readily discriminate gray matter, which contains brain cells responsible for high cognitive functioning, from white matter, which contains supportive cells with nutritive roles. As a rule, studies assessing brain developmental changes in gray and white matter have been cross-sectional, meaning that "snapshots" would be taken at different points in time. Images from groups of infants, pre-adolescents, young adults, and older adults would be considered representative of the brain for a given age. Since cross-sectional studies do not follow the same individuals over time the fine details of developmental changes could not be seen.

Advance: In a rare, large-scale, longitudinal, pediatric brain-imaging study, NIH investigators monitored the brain development of specific children over a long period of time. Brain developmental changes in the volume of white matter and gray matter observed in these children were analyzed and compared with similar data obtained from cross-sectional studies. The researchers confirmed earlier findings that there are steadily linear increases in white matter, the supportive cells, from the ages of 4 to 20 years. White matter increased approximately 12 percent overall, with the total volume increase slightly less in females than in males. In contrast, changes during development in gray matter, the "thinking" cells, were distinctly nonlinear and were region-specific. Increases in gray matter in the frontal and parietal lobes of the brain peak in boys at about age 12, and in girls around age 11. Another brain region, the temporal lobe, developed in a nonlinear manner, with a peak around 16 years of age, whereas the occipital region developed in a linear manner over the entire age range. The investigators also determined that the gray matter on the surface of the brain is approximately 10% larger in males than females, and that females reach brain "maturity" approximately one year earlier than do males.

Implications: The findings imply that the onset of brain maturity in some regions may be influenced by sex hormones in that brain maturity in females typically precedes that of males by one year, corresponding to the earlier age of onset of puberty. The research also implies that there are critical adolescent years during which brain shaping is still occurring; therefore, the environment and activities of teenagers may strongly influence brain development. The standardization of developmental data on normal subjects participating in large-scale longitudinal studies will greatly strengthen the ability of imaging techniques to be used as a diagnostic tool for disease or developmental disorders.

Giedd JN, Blumenthal J, Jeffries NO, et al: Brain development during childhood and adolescence: a longitudinal MRI study. Nature Neuroscience, 2(10):861-3. 1999.

A Scout's Guide to Axon Guidance

Background: During the development of the human brain, in a period when appropriate connections among the brain's neurons must be made for proper function, nascent neurons are faced with a bewildering array of potential trails to their destination. To help make correct choices, the axon, a thread-like process extending out of the cell body, forms a specialized tip, called the "growth cone." The role of the "growth cone" is to act as a scout to guide the axon through the tangled forest of the developing brain. The "growth cone" must be equipped to read the signs on the trail and guide the axon to reach its destination, which is achieved via molecular interactions with attractant or repellent proteins. There are a variety of receptor proteins, called *neuropilins*, and secreted factors, called *semaphorins*, believed to be important in axon guidance. If the scouting function of the "growth cone" fails, neurons will make inappropriate connections and/or die.

Advance: Using genetically modified mice, two groups of NIH-supported researchers eliminated a receptor on the "growth cone," called neuropilin-2. The function of this receptor is to interact with repellent axon guidance signals on other cells and turn the axon away from them. The elimination of neuropilin-2 hampers the scouting function of the "growth cone," which then fails to guide the axon to its proper target. Although the mutant mice survived to adulthood, certain parts of their brains had not made proper connections and other axonal pathways were completely missing. For example, two nerves that aid in controlling eye muscle movements normally extend toward their targets in a tight bundle. In mice lacking the neuropilin-2 receptor, designated as neuropilin-$2^{-/-}$ mice, the nerves extend in a branched, undefined manner. Affected pathways included those in the hippocampus, thalamus and anterior commissure. Deleting another member of the *neuropilin* family, neuropilin-1, which is found for the most part in cell populations distinct from those which express neuropilin-2, also had adverse effects on brain development. These findings clearly establish a critical role for these proteins in axon guidance. Interaction amongst various *neuropilin* receptors and the protein that binds to them is very specific. Neuropilin-1, for example, preferentially interacts with semaphorin-3A, rather than semaphorin-3F. As further evidence of the selectivity of *neuropilin* function, the investigators found that the axons that express neuropilin-1 are unaffected in the neuropilin-$2^{-/-}$ mice. The continued presence of neuropilin-2 in adult rodent brain after axon guidance events are complete suggests that this molecule may play a role in plasticity in adulthood as well.

Implications: Learning the basic mechanisms by which nerves reach their targets is essential to developing nerve-related therapeutics. Thus, it is critical to identify which factors are nerve attractants or nerve repellants and which receptors respond to them. Ultimately, this knowledge can be applied to understanding developmental disorders, illnesses involving nerve degeneration, or injury-related problems such as nerve transection.

Giger RJ, Cloutier JF, Sahay A, et al,: Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron, 25(1):29-41. 2000.

The Organization of Memories in the Hippocampus

Background: In premodern times, philosophers and scientists superimposed a figure of a human, called a *homunculus*, upon a representation of the brain as a means of depicting the association of a specific area of the brain cortex with specific sensory sensations or motor movement. More recently, regions of the human brain responsible for receiving sensory input and controlling motor movements were mapped during neurosurgical procedures for epilepsy or other brain disorders. Doctors were able to stimulate parts of the cortex and receive verbal feedback from patients as to the sensation they perceived. Structures that lie deeper in the brain, below the cortical surface, are more difficult to probe, leaving their neuroanatomical organization and function somewhat of a mystery. One particular structure, the hippocampus, is known to be one of the highest processing areas in the brain and, appropriately, critical in memory consolidation. Scientists are limited, however, in studying hippocampal function because the architecture of the hippocampus, as it relates to function, is relatively obscure.

Advance: In this research, NIH-sponsored investigators systematically placed electrodes along the hippocampal surface in rats. While the rats performed short-term memory tasks, such as pressing a left or right lever, multi-electrode recordings were obtained from groups of hippocampal neurons. Some cells were found to be "position" sensitive – that is, they fired when the rat pressed either a left or a right lever. Others were "phase-sensitive" cells that fired during a given phase (or period) of the memory exercise test, irrespective of the lever position. Yet other hippocampal neurons were classified in terms of combinations of the above features.

"Position" and "phase" cells have anatomically distinct locations. Although there were fewer position-sensitive cells, they were distributed across the largest longitudinal segments of the hippocampal structure. The cells responsive to a "left response" were anatomically distinct from "right response" cells. "Phase response" cells were identified in discrete clusters at distinct intervals, and were localized separately from "position" cells. Such anatomical separation appears to maximize the encoding of task-related and trial-specific events by ensuring that cell groups encoding different features do not overlap.

Implications: The hippocampus in humans appears to be critically important in our ability to recall personal experiences and to integrate those experiences into a bigger picture. In lower- level mammals, the hippocampus appears to be most important in learning spatial relations and applying that knowledge as it relates to cues in the environment. The discovery of the anatomical arrangement of the hippocampus, in terms of the location and distribution of cell types, provides the basis for functional differentiation. Knowledge of the functional anatomy will be useful in terms of identifying hippocampal-related pathophysiology and learning disorders.

Hampson RE, Simeral JD, Deadwyler SA: Distribution of spatial and nonspatial information in dorsal hippocampus. Nature, 402(6762):610-14. 1999.

How Fear-Related Memories are Stored – and Can Be Lost

Background: For some time, scientists have known that a brain structure called the amygdala is important in storing fear-related memories. Individuals who sustained injuries to this brain region do not recognize fearful faces and have inappropriate fear-related responses. To understand such responses and to study memory formation and fear, investigators use animal models ranging from snails to rats. A snail's "gill withdrawal" reflex, for example, can be used to assess memory of noxious stimuli. Similarly, a mild foot shock following a conditioned stimulus, such as a tone, can be used to assess memory in rats. Many of the mechanics involved in the storage of memories also have been elucidated. For instance, new memories are known to be typically fragile and subject to disruption until they are consolidated into stable, long-term memories. Research has shown that forming short-term memories requires changes in existing proteins found in brain cells, or neurons. Long-term memory formation, on the other hand, not only entails changes in existing proteins, but also requires that new proteins be made.

Advance: In recent research, investigators used traditional experimental protocols – pairing a sound (tone) with a mild foot shock – to study memory consolidation and retrieval in rats. When the researchers again sounded the tone 24 hours after training the rats, a measure of the fear response could be obtained by assessing "freezing" behavior, a common signal of fear in rats. All animals demonstrated equivalent fear responses to the tone. But, when a protein synthesis inhibitor – an antibiotic – was injected into the amygdala region following the second tone, the animals no longer elicited a fear response to the tone 24 hours later, indicating that the animal forgot to "fear" the tone. When new protein synthesis was inhibited 6 hours following the second tone, however, the animals did not "forget" to show a fearful response at the following test time. These findings suggest that protein synthesis is important in memory reconsolidation early after a memory has been retrieved.

To learn if the fear response was long-lasting, the investigators tested the animals' fear responses 14 days after the original tone and foot stimulus. Again, the rats demonstrated an appropriate freezing response to the tone. Again, too, when the protein synthesis inhibitor was injected into the amygdala region immediately following the memory test 14 days later, the animal "forgot" to freeze in response to the tone 24 hours later (at day 15). Apparently, protein synthesis is critical in the memory retrieval and reconsolidation process, even after long periods of time.

Implications: This research substantially increases the knowledge base about how fear-related memories are either "forgotten," maintained, or strengthened. Although the full implications of the work are not clear at this time, the discovery that protein synthesis is essential for the retrieval and continued remembrance of fear-related events suggests that the brain constantly undergoes structural changes. These findings lead one to conclude that "fragility" is not limited to new memories, but that any active memories are subject to disruption and require protein synthesis-dependent reconsolidation, if memory integrity is to be maintained following retrieval. Commentators on this study – but not the researchers themselves – have speculated that one clinical implication of the new information is that traumatic memories may be erasable.

Nader K, Schafe GE, LeDoux JE: Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature, 406(6797):722-26. 2000.

Experience and Biology Mold Capacity for Memory

Background: A brain structure called the hippocampus is particularly important in learning and memory-related functions. Humans with hippocampal damage have memory deficits as related to people, places, objects, and events. Animals with hippocampal damage similarly demonstrate spatial and non-spatial memory problems. One specific receptor, called the NMDA receptor, is thought to play a critical role in hippocampal-related learning and memory. With the huge variety of brain transmitters, however, which are responsible for cell-to-cell communication, and an equally large number of receptors that respond to the transmitters, it is difficult to determine the specific role and importance of any particular receptor.

Advance: NIH-funded investigators developed "knockout" mice that lack the NMDA1 receptor in one specific region of the hippocampus. Although the "knockout" mice were just as motivated, curious, and interested in exploring new objects as the "normal" mice, on tests of behavioral memory, they did not perform well. Mice normally prefer to spend more time exploring novel objects placed in their environment than familiar objects. The "knockout" mice did not seem to discriminate between a novel object and a previously explored object. Similarly, mice typically develop a preference for foods they have recently smelled on the breath of other mice. But, after "knockout" mice spent time with other mice fed with cinnamon or cocoa-scented foods, they showed no preference for these foods, indicating that their olfactory memory was impaired. They also were impaired in learning based on cues from their surroundings, or so-called contextual learning. Typically, if a mouse is exposed to an aversive stimulus in a specific environment, placing the animal in the same environment 24 hours later will elicit a freezing response. This normal, context-related response was not seen in the "knockout" mice after a 24-hour delay. The investigators found, however, that placing the mice in an enriched environment filled with running wheels, tunnels, and mouse toys promoted increases in the density of hippocampal synaptic connections in both the "knockout" and regular mice. This finding indicates that the NMDA1 receptor is critical in hippocampus-dependent, non-spatial memory, but is not essential for experience-induced, synaptic structural changes.

Implications: Information gained from this work will contribute to our fundamental knowledge of the molecular mechanisms involved in learning and memory. While the research showed that the NMDA1 receptor is vital for hippocampal-related learning, the finding that an enriched environment not only enhances learning and memory in all animals, but also can rescue deficits in challenged "knockout" animals, is exciting. The work reinforces appreciation that while genetics predispose individuals to certain learning and behavioral outcomes, the brain can be molded by experience. Therefore, nurture can modify what genetics has (or has not) given.

Rampon C, Tang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ: Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nature Neuroscience, 3(3):238-44. 2000.

Imaging Shows the Brain is a Pictionary Plus

Background: One of the most remarkable features of our visual recognition system is that we can instantly identify an object, even when we have never seen that specific object before. One explanation for this is that we have information stored in our brains, similar to a mental picture dictionary, about the visual form of different objects. We can recognize a "dog" or a "hammer" that we have not encountered before because past experience has taught us about the appearances of these objects.

Advance: Results of recent functional brain imaging studies suggests that this answer is only partially correct; information about objects is stored in a set of regions that also identify how an object moves or how it is used. The findings suggest that *information* about the features and attributes of objects are stored in different regions of the brain, close to the areas involved in the *perception* of those features.

When subjects verbally named specific objects, a number of different regions of the brain became active, depending on the object's features and attributes. Naming a picture of an animal – for example, a dog – was associated with activity in two specific areas of the brain. One region activated during the perception of visual *form*, and the other during perception of biological *motion*. In contrast, naming a picture of an inanimate object (e.g., a hammer) also produced activity close to the brain area activated during perception of *form*, but *distinct from* the region activated when naming animals. In addition, verbal naming was associated with activity near the regions activated during object *motion* perception, and when manipulating an object with the right hand. These regions were also activated when subjects read the names and answered questions about these objects.

Implications: These findings are important for a number of reasons. For example, they help to explain why some patients with focal brain damage can lose the ability to name and retrieve information about a single category of objects, such as animals or tools. The findings also suggest that information about object features and attributes are stored in different regions of the brain, close to the areas involved in the perception of those features. In a broader sense these findings provide important clues about how information necessary to understand the meaning of objects and words is stored and organized in the brain.

Chao LL, Haxby JV, Martin A: Attribute-based neural substrates in temporal cortex for perceiving and knowing about objects. Nature Neuroscience, 2(10):913-19. 1999.

Chao LL, Martin A: Representation of manipulable man-made objects in the dorsal stream. NeuroImage, 12(4):1-7. 2000.

Estrogen Increases Memory-Related Brain Cells in Adult Animals

Background: Estrogen is touted to have benefits that range from reductions in osteoporosis to heart disease. Now, research also suggests that it may increase the number of memory-related brain cells in the female rat brain. The hippocampus is an area of the brain which is particularly important in spatial-related memories. An individual with damage to this area of the brain may not be able to learn how to find his or her way home in a new neighborhood. Similarly, s/he may not be able to put past experiences into a context relevant for the present. Recent examination of normal brain function has yielded evidence of sex-related differences in hippocampal structure and function. With respect to structure, males have a greater number of brain cells and connections than do females in the dentate gyrus, an area of the hippocampus where new neurons are produced. With regard to function, females perform better on spatial learning tasks than males once novelty is taken into consideration. In the research reported here, NIH-sponsored investigators examined whether sex differences exist in the hippocampus and whether estrogen plays a role.

Advance: In seeking to ascertain the presence of sex-related differences in the hippocampal brain regions of males and females, scientists labeled and were able to detect new cell growth in the hippocampus of rats with a technique called BrdU labeling. Two days after BrdU application, female rats had 45% more BrdU-labeled cells (indicative of new cell synthesis) in the dentate gyrus region, than male rats; however, there was no difference in the total number of labeled cells 14 days after BrdU application, indicating that the females' increases in total cell number is transient, or cyclic. The BrdU-labeled cells were primarily neurons, not other types of brain cells. No sex-based differences were seen in BrdU-labeled cells in hippocampal regions other than the dentate. The researchers also found that the total number of BrdU-labeled cells in the dentate gyrus varied according to the phase of the rats' estrous cycle. Female rats had approximately 50% more new cells when BrdU was applied during proestrous (high estrogen phase of their cycle), than when it was applied during estrus or diestrus (lower estrogen) conditions. High estrogen not only seems to promote new cell synthesis in the dentate gyrus, but it also appears to prolong cell survival. Still, the increased number of new cells and extended life is short-lived; the cells give way to degeneration. Even when BrdU application occurred during high estrogen conditions, no difference exists in terms of cell number between the male and female animals 21 days later.

Implications: These findings not only confirm previous studies demonstrating that the adult brain can produce new brain cells, but also provide new evidence suggesting that structurally related sex-differences in the brain could account for variations between males and females in the processing of information and problem solving. Their data provide new insight into the importance of estrogen to females in structural remodeling of the hippocampus and imply that post-menopausal estrogen decreases could have a deleterious effect on hippocampal-dependent learning.

Tanapat P, Hastings NB, Reeves AJ, Gould E: Estrogen stimulates a transient increase in the number of new neurons in dentate gyrus of the adult female rat. The Journal of Neuroscience, 19(14):5792-5801. 1999.

SLPI is Essential for Normal Wound Healing

Background: Each year, more than four million people are afflicted with chronic non-healing wounds such as diabetic ulcers, bed sores, venous ulcers, and acute nonhealing wounds in the elderly. These impaired healing states are characterized by tissue destruction and, often, bacterial infection, leading to the hypothesis that SLPI (Secretory Leukocyte Protease Inhibitor) may play a major role in this process. SLPI is a peptide with anti-inflammatory, anti-viral, anti-fungal, and anti-bacterial properties that is found in fluids that bathe mucosal surfaces, such as bronchial fluids, cervical fluids, and saliva. It is also expressed during normal skin wound healing.

Advance: NIH scientists have produced a new animal model for delayed wound healing. These SLPI knockout mice – mice that lack the SLPI gene – show markedly impaired skin wound healing with an increase in inflammation and in elastase activity that leads to tissue destruction. The mouse model has enabled the researchers to study the complex interactions involved in aberrant wound healing and to document that SLPI plays a crucial role in normal wound healing. Topical application of SLPI reverses the abnormal response and enhances the rate of healing. Its absence leads to a condition comparable to a chronic wound.

Implications: The generation of this animal model for delayed wound healing makes it possible to study the pathological processes involved in chronic non-healing wounds. It also enables researchers to assess interventions to prevent the occurrence of these debilitating and painful conditions.

Ashcroft GS, Lei K, Jin W, et al: Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nature Medicine, 6(10):1147-53. 2000.

Cloning Resets the Telomere Clock in Cattle

Background: The potential for cloning animals depends in part on whether the procedure can reverse cellular aging and restore somatic cells to a phenotypically youthful state. Telomere shortening during cell proliferation is one cause of cellular aging, so telomere length is an indicator of the proliferative age of the cell. As the telomere length gets critically short, proliferation stops entirely, and the cell is referred to as senescent. Thus, an important question is what happens to telomere length of nuclear DNA after introduction of a nucleus into an enucleated egg.

Advance: A recent report showed that nuclei from senescent bovine fibroblasts, when transferred into enucleated egg cells, are reactivated and eventually produce healthy calves. Most importantly, these calves have longer telomeres than normal cattle the same age, and fibroblasts taken from these calves show above average proliferative potential compared to fibroblasts from the normal cattle. These results demonstrate that telomere length can be "reset" in the embryo and/or fetus in cattle, and can also be lengthened beyond the normal germ cell length. Whether this will have an impact on the life span of these cloned calves is not yet known, and will not be known for many years. Similar results have been reported (but not yet published) for mice, but not sheep.

Implications: These results suggest that the offspring in some, if not all, species of cloned animals will not be biologically older than normal offspring. They also suggest that this system may be useful for studying how telomere length is regulated. Such information will be useful in developing cell replacement intervention strategies dependent on continuing cell proliferation.

Lanza RP, Cibelli JB, Blackwell C, et al: Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. Science, 288(5466):665-9. 2000.

Genetically Mimicking Caloric Restriction Significantly Extends Yeast Life Span

Background: Aging in budding yeast is measured by the number of mother cell divisions that occur prior to cell senescence and death. Caloric restriction (CR) has been shown to significantly extend life span in a wide variety of organisms including budding yeast. Yeast cells grown on 0.5% glucose media (CR) exhibit a longer life span than cells grown on 2% glucose media. Glucose enters yeast cells via highly regulated glucose-sensing transporters and phosphate is added by the enzyme hexokinase. Elevated glucose levels in the cell activate the yeast cAMP dependent protein kinase (PKA) signaling pathway. The major goal of this research was to determine if genetic manipulation of glucose availability, metabolism, and signaling pathways could mimic the longevity-extending effects of caloric restriction in budding yeast.

Advance: Limiting glucose availability to yeast cells by mutating the hexokinase enzyme significantly extended both mean and maximum life span. Mutations in several proteins in the cAMP-dependent PKA signaling pathway that result in its reduced activity also lengthened life span. Growth of these long-lived mutants in 0.5% glucose media did not further extend life span suggesting that low glucose concentration and low PKA activity function in the same pathway to extend yeast longevity. In contrast, a mutation in the phosphodiesterase enzyme of the PKA pathway, which results in increased PKA activity, shortened life span.

Implications: In the varied organisms studied to date (yeast, nematodes, fruit flies, mice and rats) caloric restriction increased both mean and maximum life span, and health span. In all types of animals examined, the extended longevity and health of the animals was accompanied by changes in the regulation of energy metabolism. The discovery that the effects of caloric restriction can be mimicked genetically in yeast at the levels of glucose availability, glucose metabolism and signal transduction make the yeast model of aging and longevity an extremely powerful tool for dissecting the underlying cellular and molecular mechanisms responsible for increased longevity and health span.

Lin SJ, Defossez PA, Guarente L: Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science, 289(5487):2126-8. 2000.

Use of Gene Expression Microarrays in Aging Research

Background: The use of microarray high density "chips" has recently emerged as a powerful way to examine the patterns of expression of thousands of genes in a single experiment. Aging is normally accompanied by changes in expression of a large number of genes, but it is not clear which of these changes are critical in the aging process. Microarrays provide an opportunity to obtain a more complete picture of what these changes are, and to design tests of whether these changes are causally associated with aging.

Advance: Three recent publications by investigators using this technology are beginning to provide some important insights into the aging process. The first paper probed differences in transcription patterns in mouse skeletal muscle from young vs. old mice, and from untreated old vs. calorically restricted old mice. The second paper followed a similar strategy in examining transcription patterns in mouse liver. The third paper reported data on age-related changes in gene activity patterns in mouse brain. Although the data analysis is complex, some initial observations are that: 1) many, but not all, age-related changes in mouse liver and skeletal muscle are attenuated by caloric restriction; 2) aging results in lower levels of metabolic and biosynthetic genes; 3) caloric restriction appears to increase the expression of genes for repairing and/or preventing damage to cellular macromolecules; 4) aging is accompanied by transcription patterns indicative of stress responses, including inflammatory and oxidative stress.

Implications: While these conclusions are still preliminary, these papers represent a new and very efficient approach to answering long-standing important questions about the molecular mechanisms of aging. They also suggest that gene expression microarray analysis may eventually provide useful biomarkers of aging.

Lee CK, Klopp RG, Weindruch R, Prolla TA: Gene expression profile of aging and its retardation by caloric restriction. Science, 285(5432):1390-3. 1999.

Han ES, Hilsenbeck SG, Richardson A, Nelson JF: cDNA expression arrays reveal incomplete reversal of age-related changes in gene expression by calorie restriction. Mechanism of Ageing Development, 115(3):157-74. 2000.

Lee CK, Weindruch R, Prolla TA: Gene-expression profile of the ageing brain in mice. Nature Genetics, 25(3):294-7. 2000.

Extension of Average Life Span of Nematodes by Pharmacological Intervention

Background: It is widely accepted that oxidative stress is a factor in aging. However, no convincing results have been obtained demonstrating that natural anti-oxidants such as vitamins C and E, or _-carotene, extend life span in experiments with animal models such as mice, fruit flies or nematodes. Varied results have been obtained in genetically altered fruit flies over-expressing either superoxide dismutase (SOD) or SOD and catalase; these enzymes reduce oxidative damage.

Advance: A series of artificial compounds which possess both SOD and catalase activity in the same molecule were analyzed for their effect on life span. One of these, EUK-134 has been shown to increase the average life span of nematodes by about 50%. Similar results have been obtained with EUK-8, which has less catalase, but equivalent SOD activity. These compounds have no obvious effect on either fertility or body size at levels that extend life span. EUK-134 has also been shown to reverse premature aging of a nematode strain subject to elevated oxidative damage due to a mutation in one component of the electron transport chain. These results strongly suggest that oxidative stress is a major factor in the rate of aging in the nematode, and that this can be attenuated by pharmacological intervention.

Implications: It can be presumed that similar compounds might attenuate oxidative stress in humans, and in so doing delay or reduce age-related pathology. Preliminary positive effects have been observed in mice sensitive to oxidative stress.

Melov S, Ravenscroft J, Malik S, et al: Extension of life-span with superoxide dismutase/catalase mimetics. Science, 289(5484):1567-9. 2000.

Further Evidence that Presenilin-1 May Be One of the Major Amyloid-_ Forming Enzymes

Background: Amyloid precursor protein (APP) is a transmembrane protein. Amyloid beta (A_) is a peptide fragment which is produced in several forms as a result of enzymatic snipping (cleavage) of the much larger APP protein. In Alzheimer's disease (AD), abnormal forms of the peptide build up early in the disease process in brain regions involved in memory and cognition. Cleavage of APP into A_ occurs as a result of the action of enzymes called "secretases." Gamma (_)–secretase is an enzyme associated with one of the two cleavage events needed for the release of the A_ peptide, but its identity has been uncertain. Portions of the APP protein traverse both the exterior and interior of the cell, or are embedded within the plasma membrane. Because the cleavage of APP occurs within the plasma membrane instead of within the cell body itself, the _ secretase is a member of a small, as yet little understood, class of enzymes that can cleave the part of a protein embedded in membranes. While we have known that the _-secretase enzyme exists, just recently data have emerged suggesting that _-secretase may, in fact, be the protein called presenilin 1 (PS1). This is important because PS1 is mutated in some forms of early onset AD and could therefore directly cause abnormal production of the A_ protein and consequently elevated levels of A_. Several different lines of research have shown that mutations in PS1 do affect APP processing to form A_. Previous work had established that mutating two amino acids in PS1 eliminates _-secretase activity, resulting in lowered cellular production of A_. On the basis of this and other evidence, it was suggested that PS1 is actually the _-secretase itself, although the possibility that PS1 was an essential component of the reaction, rather than being the enzyme itself was not eliminated.

Advance: This study provides strong evidence that PS1 contains the active site of _-secretase and may be the enzyme itself. The team engineered a compound to directly interact with the active site of _-secretase. Strikingly, this engineered molecule specifically bound to PS1 and inhibited A_ formation in cells cultured in a test tube.

Implications: This is a highly significant finding because as a result of this study a drug might be developed as a therapeutic agent to prevent overproduction of A_ by inhibition of the action of PS1/ _-secretase. Ultimately these findings may lead to the discovery of ways to prevent both the overproduction of the amyloidogenic A_ peptide and the development of clinical Alzheimer's disease.

Esler WP, Kimberly WT, Ostaszewski BL, et al: Transition-state analogue inhibitors of _-secretase bind directly to presenilin-1. Nature Cell Biology, 2:(7):428-34. 2000.

Neuropathology in Mice Expressing Mutant Tau Protein

Background: Neurofibrillary tangles (NFT) are intraneuronal bundles of insoluble filamentous structures found inside nerve cells. They are found in a variety of human diseases including Alzheimer's disease, corticobasal degeneration, progressive supranuclear palsy and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), together called tauopathies. The NFT consist largely of an abnormal form of the microtubule-stabilizing protein tau. Tau is essential for normal cell function because it stabilizes microtubules. Microtubules play essential roles in development of cell processes and intracellular transport. There are six different forms of the tau protein (isoforms) that differ in the presence or absence of particular structural domains. Recent advances show that inherited mutations in the gene which encodes tau cause some cases of FTDP-17. These mutations can alter the structure of the protein to an abnormal one, or change the ratio of one normal protein isoform to another. These changes cause tau to fall off microtubules and make it easier for tau to aggregate into the NFTs, causing nerve cell death. Thus, tau dysfunction can directly result in neurodegeneration. Scientists have been modeling the effects of these mutations in cells in tissue culture. The present study is the first in an animal model.

Advance: Expression of one of the isoforms of human tau containing the most common FTDP-17 mutation (P301L) in transgenic mice results in both motor and behavioral deficits. Development of NFT occurred in an age- and gene-dose- dependent fashion beginning as early as 4.5 months of age (young mice) in homozygous animals. Other neuropathological features that the mice demonstrated included neuron loss and neuronal insoluble particles similar to those found in Pick's disease (Pick bodies). The distribution of these pathologies was similar but not identical to some forms of FTDP-17 and other tauopathies. For example, Pick-like bodies and NFT were expressed in brain regions such as limbic structures, midbrain, pons, medulla, deep cerebellar nuclei and spinal cord. Areas with the most NFT showed reactive gliosis (brain astrocytes become bigger and their processes enlarge in response to brain injury).

Implications: These studies link neurofibrillary pathology to neuronal loss in a transgenic animal model. Changes in tau structure are linked to loss of function and neurofibrillary pathology, for the first time allowing the key events in this neurodegenerative cascade to be examined *in vivo*. Furthermore, this work offers a system in which the relationship of neurofibrillary degeneration to other neuropathologies such as A_ production can eventually be analyzed. Finally they provide a potential model for gene or pharmacological therapy for the tauopathies.

Lewis J, McGowan E, Rockwood J, et al: Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nature Genetics, 25(4):402-5. 2000.

Transgenic Mice Expressing Human Alpha Synuclein Have Motor Impairment

Background: Synuclein, a presynaptic protein, has been found to accumulate abnormally in senile plaques in Alzheimer's disease, in Lewy body disease, and in Parkinson's disease. One form of this protein, _ synuclein, has been increasingly implicated in the pathogenesis of several of these neurodegenerative diseases which are now classified as synucleinopathies. A mutation in the _-synuclein gene has been found to be the cause of a rare form of familial Parkinson's disease (PD), sparking increased interest in the function of this protein and how changes in its structure can cause PD. Therefore, developing transgenic models carrying the _-synuclein mutation or expressing different levels of _-synuclein will help us to understand how the synucleinopathies develop.

Advance: Scientists have developed a genetically engineered mouse to examine the role of _ synuclein in neurodegeneration. This type of transgenic mouse over-expresses human _ synuclein itself; and neuropathology is seen shortly after puberty in these mice (2 months of age). The protein was predominantly deposited in the cortex and hippocampus, regions essential to cognitive function, learning, and memory. The mice had problems with motor function that were similar to those found in Parkinson's disease. They lost dopaminergic synapses in the substantia nigra, the brain region known to be most vulnerable in Parkinson's disease where dopaminergic neurons are progressively lost during the course of the disease. The workers concluded that accumulation of abnormal amounts of the _ synuclein protein may cause changes in both the architecture and function of particular neurons.

Implications: This study is important because changes seen in the transgenic mice suggest that increased amounts or accumulation of _ synuclein within neurons may play an important role in the development of pathology seen in Parkinson's disease and related disorders such as the Lewy body disease. This work offers a system in which the relationship of _-synuclein and these diseases can be studied. Finally this study provides a potential model for gene or pharmacological therapy for the progression of Parkinson's disease and Lewy body disease.

Masliah E, Rockenstein E, Veinbergs I, et al: Dopaminergic loss and inclusion body formation in a -synuclein mice: implications for neurodegenerative disorders. Science, 287(5456):1265-9. 2000.

A New Model of Parkinson's Disease

Background: Parkinson's disease is a common age-dependent and chronic progressive neurodegenerative disorder characterized by selective death of neurons that make the neurotransmitter dopamine in a region of the brain called the substantia nigra. Loss of these neurons results in movement problems including rigidity, tremor, slowed movement, and impaired gait. Besides neuronal degeneration, another hallmark pathological feature of the disease is the formation of cytoplasmic inclusions, called Lewy bodies, in neurons. These fibrous protein deposits, believed to interfere with normal neuronal function, contain _-synuclein, ubiquitin and other proteins. Studies have identified mutations in the gene encoding _-synuclein in some families in which Parkinson's disease is inherited, and it has been suggested that these gene mutations lead to altered _-synuclein proteins, which might enhance protein aggregation and the formation of Lewy bodies. To determine the function of normal and mutant _-synuclein in dopamine neurons, generation of animal models that mimic many aspects of Parkinson's disease will be required. Transgenic mice have been generated which make wild-type (normal) and mutant _-synuclein protein, and although some characteristics of Parkinson's disease are apparent, these mouse models do not exhibit all the hallmarks of human Parkinson's disease.

Advance: Scientists have developed a new transgenic model using the fruit fly *Drosophila* that exhibits many of the essential features of human Parkinson's disease. They have produced transgenic fly lines making normal human _-synuclein and separate lines making each of two mutant proteins linked to familial Parkinson's disease, A30P and A53T _-synuclein. All transgenic lines showed marked age-dependent loss of dopamine neurons in certain anatomical areas of the brain, and some neurons accumulated intracellular aggregates composed of _-synuclein filaments that resembled the Lewy bodies found in Parkinson's disease patients. This pathology is not seen in normal aged flies. Some transgenic flies also expressed a progressive, age-dependent loss of motor function as measured by climbing activity. Flies making wild-type _-synuclein or the A53T mutant performed similarly in this motor task, but locomotor deficit was more severe in flies expressing the A30P _-synuclein mutation. This suggests that the A30P mutation is more aggressive than the A53T one, but the possibility that this was due to small differences in _-synuclein protein levels was not completely eliminated.

Implications: The _-synuclein transgenic Drosophila model appears to mimic many essential features of human Parkinson's disease, including age-dependent onset, chronic progressive loss of dopamine neurons and motor function, and development of Lewy body-like pathology. This model will be useful for delineating underlying mechanisms mediating _-synuclein toxicity in dopamine neurons and to identify genetic modifiers (enhancers or suppressors) of the _-synuclein mediated neurodegeneration, which may implicate new proteins in the pathogenesis of Parkinson's disease. These transgenic flies may also provide an invaluable model to screen potential drugs affecting the onset and progression of Parkinson's disease.

Feany MB, Bender WW: A *Drosophila* model of Parkinson's disease. Nature, 404(6776):394-8. 2000.

Mad Cow Disease: The Cause of Human Fatal Neurodegenerative Disease?

Background: A class of neurodegenerative diseases, the transmissible spongioform encephalopathies, is caused by alteration of a normal protein (PrP^{C}) that is converted into a differently shaped protein (PrP^{Sc}) which is called a prion. Human prion diseases include Creutzfeld-Jakob disease (CJD) and a new variant of CJD (nvCJD). Their symptoms include a rapidly progressive dementia. They are invariably fatal. Animal prion diseases include bovine spongiform encephalopathy (BSE or "mad cow disease"). There is concern that nvCJD is caused by ingestion of beef contaminated with BSE. How this occurs has been a puzzle, but it now has been shown that PrP^{Sc} acts as a template for the conversion of PrP^{C} into more PrP^{Sc}, supporting the hypothesis that the species-specific PrP^{C} is necessary for both transmission and pathogenesis of prion disease, thus forming a 'species barrier.' However, there is a growing concern that this barrier may have been breached with the passage of BSE from cattle to humans resulting in a new variant of CJD, nvCJD.

Advance: It has been demonstrated that the transmission of BSE to mice can be accomplished by generating transgenic mice which have had mouse PrP^{C} removed and replaced with bovine PrP^{C}. These susceptible transgenic mice provide a valuable bioassay permitting accurate determination of BSE prion levels in brain and other tissues. Importantly, the new human nvCJD prion produces disease in these transgenic mice that is indistinguishable from that caused by the BSE prion in terms of incubation period, clinical course, neuropathology, localization and regional distribution in the brain, and conservation of a characteristic BSE-PrP^{Sc} fragment. The marked similarities between properties of the human nvCJD and BSE in these transgenic mice provide compelling evidence for a causal link between these prions, and strongly suggest that the human disease is transmitted by infected cattle.

Implications: The association of a new form of prion disease in humans (nvCJD) with BSE is based upon several methods: epidemiology, a comparison of protein structure, and transmission to other species. These new findings indicate that prion protein from nvCJD cases produces, in the appropriate host, disease that is indistinguishable from BSE. This indicates that the normal barrier to cross-species transmission of prion disease may have been partially breached in the case of BSE. Given the large number of cattle that have died of BSE in Great Britain, and the potential for spread into the human population, the ability to test and develop interventions is critical. This new transgenic model offers an opportunity to test a variety of potential interventions.

Scott MR, Will R, Ironside J, et al: Compelling transgenic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proceedings of the National Academy of Sciences, 96(26):15137-42. 1999.

New Neurotrophic Factor for Brain Cholinergic Neurons

Background: Acetylcholine is a chemical messenger in the brain, made in cholinergic neurons found in a region of the brain called the basal forebrain region. These cholinergic neurons connect to brain regions involved in higher cognitive functions, and disruption of these circuits leads to deficits in learning and memory. Loss of brain cholinergic function is one hallmark of Alzheimer's disease, and current therapy for this devastating disease is based on drugs that increase brain levels of acetylcholine. Several neurotrophic and growth factors have been identified that promote survival and growth of cholinergic neurons, and maintain levels of critical proteins involved in acetylcholine production. A family of growth factors, called bone morphogenetic proteins (BMPs), has recently been shown to affect nervous system development by, for example, promoting the survival and maturation of neurons.

Advance: This study showed that BMP-9, a relatively uncharacterized member of the BMP family, was made in brain areas of fetal mice and increased production of cholinergic properties in immature neurons. Treatment of fetal brain cells grown in culture with BMP-9 increased acetylcholine content in cells in a time and dose dependent manner, and stimulated production of proteins, such as choline acetyltransferase, which are needed for the synthesis of acetylcholine. Injection of BMP-9 into brains of mouse embryos also increased acetylcholine levels, though this response was reduced when injected into newborn mouse brain.

Implications: These results indicate that the maturation rate of cholinergic neurons may be enhanced by BMP-9 and suggest a model in which local synthesis of BMP-9 in the vicinity of the developing cholinergic neurons acts to increase the expression of key proteins needed for optimal cholinergic cell function. Although additional research, particularly in animal models of brain disorders is needed, these results suggest that BMP-9 may have potential use in the treatment of neurological disorders in which cholinergic neuron function is compromised.

Lopez-Coviella I, Berse B, Krauss R, Thies RS, Blusztajn JK: Induction and maintenance of the Neuronal cholinergic phenotype in the central nervous system by BMP-9. Science, 289(5477):313-16. 2000.

Study of Genetic Recombination in Malaria Parasite Will Lead to Localization of Virulence and Drug-resistance Loci

Background: The malaria parasite, *P. falciparum*, is a major cause of disease in developing counties. In the effort to combat malaria, the international research community has embarked upon a collaboration with the goal of deciphering the complete DNA sequence of the *P. falciparum* genome, which contains all of its genes and other genetic information. A parallel goal is to determine the role of the genes and how they function in disease. Developing a linkage map showing gene locations and patterns of genes that tend to be inherited together, or "linked," is vital to meeting these goals and locating areas in the genome involved in virulence, drug resistance, and transmission.

Advance: Inheritance patterns for 901 genome markers – landmarks whose positions along the complete sequence of a genome are known – were established by studying 35 independent progeny from a cross between two strains of *P. falciparum*. A linkage map indicating genes that are co-inherited, and therefore likely to be close together, was constructed from an analysis of these patterns over the total 14 chromosomes of the genome. The study also revealed a high frequency of meiotic crossover in the parasite genome, where segments of DNA from each parent are exchanged, or recombined, during reproduction. In addition, a large number of gene conversions, in which a gene from one parent completely replaces the gene from the other parent in the progeny, were detected.

Implications: First and foremost, this study has provided a dense, uniform mapping framework of DNA markers which will provide a scaffold onto which the complete genome sequence of *P. falciparum* can be assembled. Such a framework enables researchers to assign chromosomal locations and relative positions of genes on the chromosome with great accuracy. Knowledge of the complete genome of *P. falciparum* – both sequence and gene locations – will make it possible to design better drugs with which to combat the malaria parasite. This study has also provided insights into how the parasite genome changes with time and how parasite populations might evolve. The finding of a high recombination rate reveals a mechanism by which the parasite is able to evolve very rapidly. The unusual number of gene conversion events detected suggests a further mechanism by which such traits as drug resistance or virulence might change quickly in response to environmental factors or human intervention.

The linkage map is made available to the research community by the NIH: at http://www.ncbi.nlm.nih.gov/Malaria.

Su X, Ferdig MT, Huang Y, Huynh CQ, Liu A, You J, Wootton JC, Wellems TE: A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum.* Science, 286(5443):1351-3. 1999.

Spotlight on Visual Proteins: Visual Protein Sees the Light of Day

Background: Most individuals are familiar with entering a darkened room and having their eyes gradually adjust to the low light levels so that they can find the light switch. At the cellular level, the ability to see objects at these low light levels is based on the binding of the chemical "retinal" (a derivative of Vitamin A) to a protein forming rhodopsin in rod photoreceptor cells in the retina of the eye. Vision begins when light causes a change in the conformation of bound retinal. A conformational change in the three-dimensional structure of rhodopsin then takes place, thereby initiating a cascade of events that results in vision. Understanding how the dynamic structure of rhodopsin acts as a trigger in the cascade of events leading to vision has been an important and difficult goal of scientists studying vision and vision loss.

Advance: Scientists have now succeeded in identifying, for the first time and at very high resolution, the three-dimensional structure of rhodopsin. This finding is significant because this level of resolution permits an analysis of the orientation of individual atoms of the protein to one another. The results confirm that rhodopsin consists of a bundle of seven helices that span the cell membrane and include both extracellular and intracellular domains that are essential to its function. Importantly, knowledge of this architecture will help scientists understand how conformational changes in rhodopsin initiate the next step in the vision cascade, the activation of a guanosine triphosphate-binding protein (G-protein). Elucidating the coupling of the rhodopsin receptor with the G-protein may provide a significant new avenue for the development of therapeutics to combat vision loss. Finally the identification of this structure in a mammalian system will bring scientists a step closer to modeling disorders involving the human rhodopsin protein.

Implications: High resolution identification of the three-dimensional structure of rhodopsin has broader implications for other biological systems besides vision. Rhodopsin is a member of the large class of G-protein-coupled receptors. Instead of responding to light, other receptors respond to stimuli such as hormones, calcium ion levels, or odorants to initiate a biological response. Regardless of the stimulus, all of these rhodopsin-like proteins are linked to specific G-proteins. Elucidation of the dynamics of how G-protein coupled receptors like rhodopsin transduce chemical information will have important implications for research in many biological systems.

Palczewski K, Kumasaka T, Hori T, et al: Crystal structure of rhodopsin: a G protein-coupled receptor. Science, 289(5480):739-45. 2000.

Seeing with Rewired Brains

Background: How do our brains organize sensory inputs? Is the development of this organization driven by intrinsic mechanism such as genetically controlled programs (nature), or by extrinsic factors such as a neural code of what we see and hear (nurture)? NIH-supported researchers have approached this question using the developing sensory systems of newborn ferrets. The ferret is an ideal animal model for this work because its brain is less developed at birth than the brains of most other newborn mammals. The approach involves surgically manipulating the sensory nerves that go to sensory centers of the brain. Nerves from the eye are misrouted from their usual target, the primary visual cortex, to the auditory cortex, which has been deprived of its normal auditory input. Work with this model demonstrated that the "rewiring" procedure resulted in the development of a functional visual cortex in a part of the brain that was otherwise destined to become the auditory cortex. In these animals, the rewired visual cortex had the visual map that is found in a normally wired visual cortex, and the nerve cells were taken over by the visual input. These experiments suggested that the organization and response characteristics of different regions of the brain can be shaped by the activity of nerve cells arising from the sensory input; in this case visual vs. auditory inputs.

Advance: More recent work has taken this thesis farther. The latest studies show that higher order features seen in a normally wired visual cortex, such as columns of cells that represent the orientation of the visual stimulus (orientation columns), are present in the rewired cortex. Further, behavioral studies on rewired animals show that they respond appropriately to visual stimuli arising from the activity of neural circuits in the rewired centers of their brains. These animal "see" with their auditory cortex.

Implications: These observations offer a direct challenge to the view that the development of brain organization is not dependent on the input activity. This research is also an important physiological extension of a long-standing philosophical view stating that the sources of inputs to the brain play a significant role in determining perception and brain function.

Sharma J, Angelucci A, Sur M: Induction of visual orientation modules in auditory cortex. Nature, 404(6780):841-7. 2000.

Von Melchner L, Pallas SL, Sur M: Visual behaviour mediated by retinal projections directed to the auditory pathway. Nature, 404(6780):871-6. 2000.

Neovascularization Associated with Age-Related Macular Degeneration

Background: Age-related macular degeneration is the leading cause of blindness in patients over the age of 65. As the population in this country ages, this disease will have an even greater impact. The condition affects the retina and leads to varying degrees of vision loss depending on the form and severity of the disease. In initial phases, the disease caused reductions in the ability to read fine print and see in dim light. In the later stages of the disease, abnormal blood vessel growth takes place under the retina and causes severe vision loss resulting in an inability to drive, read, recognize faces, and perform other visual tasks of day to day living. While the disease has been recognized for many years, our understanding of the causes and reasons for progression of this disease are still limited. One of the primary reasons for this dearth of knowledge is an absence of an appropriate animal model that mirrors the clinical presentations of the disease. Work in humans with this conditions has indicated that certain proteins involved in growth of blood vessels are elevated in these patients and that one growth factor, vascular endothelial growth factor (VEGF), is consistently elevated in patients with abnormal blood vessels associated with age-related macular degeneration. Understanding how this disease arises and develops is a major goal of many investigators and would enhance the ability to establish new methods of diagnosis and treatment for this important disease.

Advance: For the first time, scientists at the NIH, using a system to manipulate the expression of VEGF have been able to cause rodents to develop abnormal blood vessels that are identical in location and appearance to those seen in humans afflicted with the disease. This finding is important, because, to date, no animal model has been developed that mimics the findings in humans.

Implications: Modeling this condition in animals will provide an invaluable research tool to study the causes and to test treatments for this condition. Because the model takes advantage of a stimulus known to occur in the human condition, a more precise understanding of the trigger factors for the growth of the blood vessels will be gained. Subsequently, these trigger factors can then be manipulated through various therapeutic mechanisms that should be directly applicable to patient care. By understanding and using this new model, scientists hope to develop better tools to treat patients with age-related macular degeneration.

Baffi J, Byrnes G, Chan CC, Csaky KG: Choroidal neovascularization in the rat induced by adenovirus mediated expression of vascular endothelial growth factor. Investigative Ophthalmology and Visual Science, 41(11):3582-9. 2000.

Function of Osteonectin/SPARC in the Retina

Background: Osteonectin is a protein that was initially found in bone and that has been shown to be secreted by tissues throughout the body. Osteonectin is involved in cell growth, proliferation, and differentiation, and has anti-adhesive properties. Osteonectin has also been implicated in ocular disease. Osteonectin expression is increased in human age-related cataracts and a mouse model that is deficient in osteonectin develops an age-related cataract. NIH scientists have also been studying the function of this protein in the retina.

Description: The macula is the portion of the retina that provides sharp central vision. Osteonectin mRNA is expressed in 8-10 fold greater levels in the macular retinal pigment epithelium (RPE) – the tissue that supports many of the retina's metabolic functions – than in the peripheral RPE. The osteonectin protein has been detected in the macula and in the peripheral neural retina, but only traces are found in the peripheral RPE and choroid. Immunocytochemical studies have localized osteonectin mostly in the outer plexiform layer (OPL) – the part of the retina containing the axons of the photoreceptor cells – but also in the macular RPE.

Implications: These data suggest osteonectin is synthesized by the macular RPE and is then secreted and transported to the OPL. The increased levels of the mRNA in the macular RPE may be due to greater turnover of osteonectin in the macula versus the peripheral retina. The function of osteonectin in the retina is unknown, but its high levels of expression by the macular RPE and its localization to the OPL are unique and suggest that it may have an important role in macular function. Additional studies are in progress to further localize the sources of osteonectin in the retina as well as the structure(s) that it interacts with in the OPL.

Rodriguez IR, Moreira EF, Bok D, Kantorow M: Osteonectin/SPARC secreted by RPE and localized to the outer plexiform layer of the monkey retina. Investigative Ophthalmology and Visual Science, 41(9):2438-44. 2000.

Scientists Identify Malaria Gene that Confers Resistance to Chloroquine

Background: Infection by malaria parasites is responsible for more than 1 million deaths worldwide each year. Of the four species that infect humans, *Plasmodium falciparum* is the most widespread, appearing throughout much of Central and South America, Africa, and Asia. It is also the pathogen responsible for the most severe forms of disease and the highest death rates. For decades, chloroquine has been the gold standard for treatment of malaria, by virtue of the efficacy, lack of toxicity, and affordability of this drug. However, in recent years, strains of chloroquine-resistant *P. falciparum* have become widespread throughout Africa, Asia, and the Americas. This new threat to public health has proved especially severe in sub-Saharan Africa, particularly in the pediatric population, for which malaria is a leading cause of morbidity and mortality.

Advance: Scientists have now isolated and mapped a gene that has a central role in the development of chloroquine resistance in *P. falciparum*. Located on chromosome 7, the *pfcrt* gene directs the synthesis of a protein called PfCRT that performs transport functions within the parasite. Researchers confirmed the presence of a mutated *pfcrt* gene in all 24 examined chloroquine-resistant strains of *P. falciparum* that have evolved independently in different locations in the Americas, Africa, and Asia. They also verified that *pfcrt* mutation did not occur in parasite strains that remained responsive to chloroquine. Chloroquine-sensitive strains manipulated to express mutated *pfcrt* genes acquired the ability to propagate in the presence of chloroquine at concentrations tolerated only by naturally chloroquine-resistant strains. The altered PfCRT protein may interfere with the accumulation of chloroquine by the parasite as it digests hemoglobin from its human host. Alternatively, the protein may prevent chloroquine from binding to hematin, a component of red blood cells. Further research is needed before the exact physiological mechanisms leading to chloroquine resistance in *P. falciparum* are elucidated.

Implications: Identification of this gene and its product will enable scientists to better understand basic biological mechanisms of drug resistance at the molecular level. Ultimately, this knowledge may lead to the development of new antimalarial drugs that may be used either independently or in conjunction with chloroquine to reduce mortality rates. Furthermore, this discovery may allow rapid mutation-based *pfcrt* molecular assays to identify chloroquine resistant *P. falciparum* malaria.

Fidock DA, Nomura T, Talley AK, et al: Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Molecular Cell, 6(4):861-71. 2000.

Determination of the Crystal Structure of a Natural Killer Cell Inhibitory Receptor Engaging a MHC Class I Molecule

Background: Nearly every cell in the body has on its surface molecules called major histocompatibility complex (MHC) class I molecules. The MHC class I molecule plays an essential role in the immune system by presenting bits and pieces of pathogens, particularly viruses, for recognition by killer T cells. (Killer T cells are a type immune system cell that destroy their targets on contact.) The MHC class I molecules also present aberrant proteins that may be produced in tumor cells. Given that such intercellular materials would remain hidden from killer T cells if not displayed by the MHC class I molecules, it is not surprising that many viruses and tumor cells have evolved mechanisms to stop production of MHC class I molecules to avoid killer T cell responses. The immune system has countered this escape strategy by generating a class of cells called natural killer (NK) cells that monitor all cells in the body for normal levels of MHC class I expression. Cells without an adequate level of MHC class I molecules are killed by NK cells; those with normal levels of MHC class I molecules are spared.

The action of NK cells is regulated by two types of receptors – activating receptors and inhibiting receptors. NK cells are stimulated to kill cells when their activating receptors are engaged by any of an array of molecules ubiquitously present on the surface of cells. NK cells are stopped from killing cells when inhibiting receptors override, or block, the message from activating receptors. Because inhibiting receptors bind to MHC class I molecules, cells with normal levels of MHC class I molecules on their surface have the capacity, through inhibiting receptors, to signal their normalcy to the NK cell. Thus, the activating and inhibiting receptors work in concert to allow NK cells to recognize and kill any cell in the body that does not express normal levels of MHC class I molecules, indicating that the cell may be cancerous or infected by a virus. Because NK cells play a critical role in host defenses against tumor cells and some virus infected cells, there is considerable interest in exploiting the killing potential of NK cells in cancer and viral therapy.

The recognition of MHC class I molecules by NK cells is a challenge for the immune system because there are many genetic variations among MHC class I molecules. For killer T cells this does not present a problem because killer T cells have a vast set of diversified receptors with which to recognize the various MHC class I molecules. However, NK cells have only two families of inhibitory receptors, and these receptors must recognize all types of MHC class I molecules. Presumably, inhibitory receptors see only those parts of MHC class I molecules that most MHC class I variants have in common. A central element in the design of any therapy relying on NK cells is an understanding at a molecular level of how NK cell receptors see the MHC class I molecules.

Advance: Using X-ray crystallography (a method for determining the three-dimensional structure of proteins), scientists have now succeeded in determining the molecular structure of an inhibitory receptor bound to a MHC class I molecule. The bound receptor for which the investigators created a three-dimensional map is killer-cell immunoglobulin-like inhibitory receptor (KIR). The structure of the KIR-MHC class I complex clearly revealed how the KIR engages the MHC class I molecule and precisely defined the area on the MHC class I molecule that the KIR contacts. Significantly, determination of the structure also revealed the differences between NK and T cells in how they recognize MHC class I molecules, clearly distinguishing their modes of recognition for the first time. In addition, the crystal

structure provided important clues as to how the KIR may trigger the intracellular events necessary to block the activating signals once the NK cell has engaged a sufficient number of MHC class I molecules.

Implications: This work offers the first insights into how an important family of NK cell receptors binds with MHC class I molecules. Now that the area of contact between MHC class I molecules and KIR has been defined, scientists can target that area on MHC class I molecules in their drug development efforts. By revealing the molecular details of NK cell recognition, the investigators have opened new avenues of research that may ultimately reveal how to promote the activity of NK cells to kill tumor cells in cancer and infectious disease therapy.

Boyington JC, Motyka SA, Schuck P, Brooks AG Sun PD: Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class l MHC ligand. Nature, 405(6786):537-43. 2000.

Crystal Structure of Novel Protein Reveals New Treatment Target for Immune-Mediated Diseases

Background: New vaccine development will require a greater understanding of the ways in which immune system cells recognize and respond to pathogens (disease-causing agents). Special cells of the immune system, called antigen-presenting cells (APCs), ingest foreign microorganisms and then display antigens (small protein fragments of the microorganism) on their cell surfaces to activate other immune cells, e.g., T cells, to respond to the invader. The infecting pathogens may be viral, bacterial, fungal or parasitic. A protruding cell surface protein called major histocompatability complex (MHC) binds and displays the antigen. T cells (immune system cells that protect against invading pathogens) use special receptors to bind to antigen-MHC complexes. When sufficiently bound, the T cell is activated. Two types of MHC molecules are known, class I and class II. They differ in function in that class I complexes trigger cytotoxic T-cells or killer T-cells, whereas class II complexes activate helper T-cells. Both types of T cells work in concert to eliminate pathogens. Earlier research by NIH-supported investigators used a technique called X-ray crystallography to show that killer T-cell receptors bind diagonally on the surface of the antigen-MHC class I complexes. The same team of scientists has been working to reveal the binding pattern of helper T-cell receptors using the same state-of-the-art technology.

Advance: For the first time, how helper T-cell receptors orient to bind to antigen-MHC class II complexes has been revealed. The discovery was made by the same team of scientists who helped pioneer X-ray crystallography on killer T-cell receptors. Remarkably, the researchers found that helper T-cell receptors attach themselves in a perpendicular orientation rather than in the diagonal orientation exhibited by receptors of cytotoxic T cells.

Implications: The difference in receptor docking between the functionally distinct T cell subsets strongly suggests that each subset can be targeted separately by novel vaccine candidates or therapeutic methods to optimize the appropriate type of immune response. This new information is critical for vaccine design and may lead to better treatments for immune-mediated diseases and disorders such as asthma, allergies, transplant rejection, type 1 diabetes, rheumatoid arthritis, and multiple sclerosis.

Reinherz EL, Tan K, Tang L, et al: The crystal structure of a T cell receptor in complex with peptide and MHC class II. Science, 286(5446):1913-21. 1999.

Genes Provide Clues to TB Persistence

Background: Each year *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), claims 2 million to 3 million lives worldwide. *M. tuberculosis* infects an estimated one in three persons annually or about one third of the world's population. Most infected individuals will have a dormant or "persistent" infection for their entire lives, characterized by long term residence of *M. tuberculosis* in several cell types including macrophages, which are cells of the immune system responsible for ingesting foreign microorganisms. However, during their lifetime, at least 1 in 10 of persistently infected individuals will eventually become sick from TB. Despite decades of intense and productive investigation, little is known about how these persistent infections are established and controlled in most people or how, in others, these infections become re-activated and cause disease.

Advance: Two studies are providing clues to help eliminate this deadly disease. The first used a bacterium called *M. marinum*, a close relative of *M. tuberculosis* that causes a TB-like disease in marine animals. Several genes were identified that are preferentially "turned-on" when *M. marinum* resides within individual macrophages, or in the clusters of fused macrophages called granulomas that typically form in response to persistent infection. When two of the identified genes, which have very similar counterparts in *M. tuberculosis*, were experimentally inactivated in *M. marinum*, the bacteria were no longer able to divide and grow within macrophages and were less able to persist in granulomas. This finding suggests that the two genes play a role in mycobacteria's ability to cause persistent infections. A second study investigated the genetic basis for "cording" (the process by which TB bacteria produce long rope-like formations). The cording phenomenon is important because the virulence of a given TB strain appears to correlate with the strength of the strain's cording. First the investigators screened the genes of a related strain of bacteria (BCG) for mutants that fail to form cords. Then they compared those genes with the genes of *M. tuberculosis*. By this means, they discovered that an *M. tuberculosis* gene involved in the synthesis of cyclopropane ring is probably also responsible for cording. Once they had identified the function of the gene, they renamed it *pcaA*. When the investigators inactivated *pcaA* in *M. tuberculosis*, the bacteria were less able to persist and cause TB disease and death in laboratory mice. This work confirmed that the mutant BCG gene is applicable to chronic TB infection and is associated with reduced mortality. In the process of their experiments, the investigators also developed a much more efficient technique for inactivating (knocking out) bacterial genes in TB-related bacteria.

Implications: The results of both studies advance the understanding of TB infection and disease, and potentially provide targets for development of new drugs and disease prevention strategies. Furthermore, the new gene knockout technique should expedite the determination of the functions of many other TB genes.

Ramakrishnan L, Federspiel NA, Falkow S: Granuloma-specific expression of mycobacterium virulence proteins from the glycine-rich PE-PGRS family. Science, 288(5470):1436-39. 2000.

Glickman MS, Cox JS, Jacobs WR Jr: A novel mycolic acid cyclopropane synthetase is required for cording, persistence and virulence of *Mycobacterium tuberculosis*. Molecular Cell, 5(4):717-27. 2000.

Researchers Identify Ebola Virus Gene that Causes Massive Hemorrhaging

Background: The Ebola virus is one of a group of viruses that causes hemorrhagic fever in monkeys and humans. It is an extraordinarily virulent pathogen that kills up to 90 percent of infected individuals, most often by causing massive internal bleeding. The prevailing scientific view is that Ebola is maintained in an animal species (not yet identified) native to the African continent and is introduced into the human population though contact with that species. Ebola Hemorrhagic fever appears sporadically and unpredictably in certain regions of Africa, often devastating entire villages. Ebola infection causes a high fever, most frequently followed by widespread hemorrhage, and a consequent radical drop in blood pressure leading to death. Ebola virus causes hemorrhage by destroying the endothelial cells that line the interior of blood vessel walls; as these cells die off, vascular walls become thinner, weaker, and eventually begin to leak. The virus also provokes an immune-system reaction that is believed to further aggravate bleeding. No vaccine against Ebola exists; nor is there any effective cure for infected individuals.

Advance: Glycoproteins are large molecules composed of protein and carbohydrate. Investigators discovered that, when broken down inside an endothelial cell, a glycoprotein specific to Ebola causes cell death. Moreover, they isolated the major Ebola virus gene that codes for the protein portion of the glycoprotein molecule and identified the section of that gene that is responsible for the protein's ability to combine with a carbohydrate in order to produce a glycoprotein. Glycoproteins form part of the outer covering, or envelope, of a viral particle (virion). The glycoprotein (GP) identified by the investigators enables Ebola virions to attach to vascular endothelial cells and facilitates the insertion of Ebola virus genetic material into these cells. After infection, the Ebola GP gene directs the cell to begin manufacturing, processing, and transporting GP to the cell's surface. Within 24 hours, the rapid production of newly-synthesized GP causes the endothelial cells to swell and detach from blood vessel walls; these cells die within 2 to 4 days. When investigators altered the GP gene and removed the section that codes for the protein's ability to bind with a carbohydrate (the mucin domain), researchers found that GP lost its ability to destroy endothelial cells.

Implications: Identifying the gene and gene product responsible for vascular endothelial cell death may facilitate the development of effective medications to treat Ebola as well as other related hemorrhagic fevers. In addition, the more complete understanding of the structure of the GP molecule and the identification of the area of the molecule critical to protein-carbohydrate binding may ultimately lead to the development of a vaccine that will prevent the cellular processes leading to the massive hemorrhaging characteristic of this disease.

Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG Nabel GJ: Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nature Medicine, 6(8):886-9. 2000.

Snapshots of Nature: Crystal Structure of an Interaction that Triggers Asthma and Allergic Diseases

Background: Diseases resulting from immediate hypersensitivity reactions include allergy, asthma, and anaphylactic shock. The wide spectrum of symptoms range from mildly annoying to life threatening. Allergic rhinitis and asthma result from a special immune system response to common environmental allergens. These responses are initiated by immunoglobulin E (IgE) antibodies that bind specifically to the allergens. The molecular structure of IgE antibodies allows them to also bind to special receptors on mast cells and basophils, stimulating these cells to release inflammatory substances responsible for the symptoms characteristic of immediate hypersensitivity reactions: itching, swelling, bronchial constriction, and in the most extreme cases, a severe reaction requiring hospitalization. A number of drugs can blunt the effects of inflammatory mediators and partially relieve these symptoms. But no drug has yet been developed that can prevent the onset of immediate hypersensitivity reactions by interfering with the ability of IgE to bind to its receptor on cells.

Advance: Using a method of protein structure determination called x-ray crystallography, researchers created a three-dimensional map of the junction between IgE and the Fc_RI receptor (the specific receptor on the surface of immune system cells, e.g., mast cells and basophils, to which IgE binds). This has enabled scientists to determine the precise molecular interactions that take place when the IgE molecule becomes directly linked to its receptor on immune cells.

Implications: Detailed knowledge of the IgE receptor complex structure will greatly facilitate the development of new drugs to treat and prevent allergies and asthma.

Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetsky TS: Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc_RI____Nature, 406(6793):259-65. 2000.

Novel Protein on Dendritic Cells Delivers HIV to T Cells

Background: Dendritic cells provide a first-line of defense against viruses and other pathogens on vulnerable mucosal surfaces such as those in the rectum, vagina, and cervix. When dendritic cells encounter a microorganism they ingest it and display pieces of the foreign proteins on their surface to activate other immune system cells, such as T cells to attack the invading microorganism. While it has been proposed that dendritic cells localized in these areas play a role in initial HIV-1 infection, the specifics of that role have not yet been worked out.

Advance: A team of NIH-supported investigators and scientists from the Netherlands has determined that a protein, designated DC-SIGN, present on the surface of dendritic cells can bind to the surface of HIV. But this interaction does not initiate the process of ingestion and display of antigen discussed above. Rather, the DC-SIGN-HIV complex remains bound to the surface of a dendritic cell as it migrates from the mucosal lining of the vagina and rectum to the lymphoid organs to fulfill its normal function of seeking out and associating with T cells. Further, the researchers determined that the DC-SIGN-HIV interaction does not alter the infectivity of the virus. Thus, HIV can infect other cells, including T cells, when it reaches the regional lymph nodes. Because T cells are a primary target of HIV, the DC-SIGN protein actually facilitates a more efficient process of HIV infection.

Implications: The findings suggest that DC-SIGN may play a critical role in the fundamental steps of HIV-1 pathogenesis by transporting virus from the mucosal and dermis layers to remote lymph nodes. Further study of the role of DC-SIGN in HIV-1 infection may lead to better understanding of the fundamental mechanism of HIV-1 transmission and to development of strategies to prevent or block viral infection with vaccines or therapeutics.

Geijtenbeek TBH, Kwon DS, Torensma R, et al: DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. Cell, 100(5):587-97. 2000.

Resting $CD4^+$ T Cells Are Not the Only Source of Resurgent HIV Virus Following Highly Active Antiretroviral Therapy

Background: Highly active anti-retroviral therapy (HAART) involving treatment with a combination of several powerful anti-retroviral drugs has dramatically improved the long-term prognosis for HIV-infected individuals. HAART can significantly delay the onset of AIDS in HIV-infected patients by reducing the level of the virus in the body to near-undetectable levels. As a result, this innovative therapy has substantially reduced AIDS-related morbidity and mortality rates in the U.S. and other developed countries. The early success of HAART led to the initial hope that 1 to 3 years of treatment could completely clear the virus in infected individuals. However, even with HAART, HIV escapes complete eradication due to low-level replication and to its ability to "hide" within certain cell populations. After HAART treatment is discontinued, levels of HIV rapidly rise in most patients. Resting $CD4^+$ T cells were the first cell population to be identified as a reservoir for latent HIV virus. Although researchers have suspected that there must be other HIV reservoirs besides resting $CD4^+$ T cells, no firm evidence of the existence of such reservoirs had been uncovered.

Advance: Scientists have now uncovered strong evidence indicating that, in the majority of infected individuals, multiple reservoirs harbor latent HIV. By analyzing the genetic makeup of actively replicating virus in the blood plasma and in the latently infected resting $CD4^+$ T cells of several patients, the investigators determined that those pools of virus (that from blood plasma and that from $CD4^+$ resting T cells) are distinct. This evidence suggests that the resurgent virus originates from multiple sources in the body, not only from resting $CD4^+$ T cells.

Implications: This discovery will stimulate research to identify the other reservoirs of latent HIV. That research will be the first step in discovering new therapies to contain or eliminate latent viral populations and new strategies to prevent the renewal of these viral reservoirs.

Chun TW, Davey RT Jr, Ostrowski M, Justement JS, Engel D, Mullins JI, Fauci AS: Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of high active anti-retroviral therapy. Nature Medicine, 6(7):757-61. 2000.

New Red Blood Cell Mutation Associated with Resistance to Malaria

Background: Epidemiological studies have shown that certain mutation affecting red blood cells, such as the sickle cell mutation of hemoglobin, affect the frequency and severity of malaria in endemic regions. The mechanisms by which these mutations modulate malaria are not known. Most Africans lack the Duffy blood group antigen FY and appear to be protected from *P. vivax* infection, a more "benign" form of malaria, which is relatively uncommon in Africa compared to other malaria endemic areas of the world. For example, *P. vivax* is holoendemic with other forms of malaria in Papua New Guinea, where many mutations in hemoglobin and red cells are known to exist. Alpha thalassemia, one such common mutation, is associated with an increased risk of vivax malaria, and that vivax malaria may act as a "natural vaccine" to reduce the severity of subsequent falciparum malaria infection. The authors have discovered a new mutation in the Duffy blood group gene (FY*A) which renders the erythrocyte Duffy blood group negative and reduces their susceptibility to vivax malaria.

Advance: These data suggest that *P. vivax* infection is involved in the selection of red blood cell genetic variance and that the new mutation may diminish the protective effect of alpha thalassemia mutation. Further understanding of natural selection for mutations that affect the frequency and severity of malaria will help to develop novel new strategies to combat this disease.

Implications: In the long-term this mutation may compromise the alpha thassemia /*P. vivax* mediated protection against severe falciparum malaria. The identification of this novel red blood cell antigen mutation should contribute to understanding the genetic contribution to mechanisms of human resistance to severe malaria pathogenesis.

Zimmerman PA, Wooley I, Masinde GL, et al: Emergence of FY*A^{null} in a plasmodium vivax endemic region of papua new guinea. Proceedings of the National Academy of Sciences, 96(24):13973-77. 1999.

Sequencing the Mouse Genome: Providing Scientists with Tools to Interpret the Human Genome while Gaining Molecular Insight into a Powerful Model System

Background: Sequencing the mouse genome has emerged as a major priority of the Human Genome Project. Deciphering the genomic sequence of model organisms is a critical component to the interpretation of the colossal string of human genetic code and to the development of bioinformatics strategies to uncover the identity, structure, and function of genes that lie within it. A unique strength of human-mouse sequence comparisons is their ability to reveal regulatory elements in noncoding segments of the human genome, a task for which no other current computational technique is even moderately effective. These snippets of genetic sequence, often only 4-8 letters in length, play a vital role in determining where, when and to what extent, each gene will be expressed. This spatial and temporal specificity is fundamental to our knowledge of developmental, as well as many disease, processes. The mouse is one of the most significant laboratory animals for studying human disease because mice and humans share many of the same fundamental biological and behavioral processes, and a great number of mouse models of human diseases have already been identified. Mouse models provide scientists with unprecedented insights into the molecular basis of disease and the response to potential therapeutic agents.

Advance: The effort to sequencing the genome of the laboratory mouse was launched by NIH in October 1999. Ten laboratories, now referred to as the Mouse Genome Sequencing Network (MGSN), are initially focusing their efforts on achieving broad coverage of the genome while completing maps that establish the physical organization of the 21 mouse chromosomes. Sequencing from those mapped clones will then produce an intermediate working draft version of the mouse genome. As with the human genome sequencing strategy, in the second stage, attention will turn to filling any gaps in the draft and finishing the sequence in high quality, final form no later than 2005.

A novel aspect of the mouse genome sequencing strategy will be the allocation of a proportion of the sequencing capacity to targeted regions of the genome that have evidence of significant biological interest. Applications are submitted by researchers, then reviewed and prioritized by a team of reviewers assembled by NIH.

Implications: Mouse and humans are approximately 85% identical at the genetic level in coding regions of the genome. Both genomes contain approximately 3 billion base pairs and encode an estimated 50-100,000 genes. The invaluable contribution of mouse models toward a better understanding of human disease has been long recognized in biomedical research. For example, intramural scientists at NIH are developing and utilizing mouse models to study a diverse array of human diseases. These include brain disorders such as Huntington's disease, neural crest disorders, and blood disorders such as acute myeloid leukemia.

http://www.nih.gov/science/models/mouse/

Researchers Decipher the First Two Chapters of the Human Genetic Instruction Book

Background: The human genome is packaged into 23 pairs of chromosomes, often referred to as chapters in this vast genetic instruction book. While individual genes have been identified and sequenced for decades, up until the past year, scientists never had the opportunity to look at the genomic landscape of an entire chromosome. It has been compared to seeing an ocean liner emerge out of the fog, when all you've ever seen before were rowboats.

Advance: In the December 2nd, 1999, issue of Nature, an international team of researchers reported for the first time the sequencing of the 33.5 million base pairs of chromosome 22. The sequence included the longest, continuous stretch of DNA ever assembled, at over 23 million base pairs.

Just a few months later, scientists in Japan and Germany published, in the May 18th, 2000 issue of Nature, the "finished" genetic sequence of human chromosome 21. Studying the organization of the genes on chromosome 21 and how they and their protein products function will help scientists find clues about Down syndrome as well as other disorders, including Alzheimer disease, certain cancers, and manic depressive illness, which have also been linked to this chromosome. With the complete DNA sequence of chromosomes 22 and 21 now in hand, scientists can begin to study structural similarities between and among chromosomes as well as shared sequences.

Implications: The next mammoth task is to determine what it all means. Sequencing and mapping efforts have already revealed that chromosome 22 is implicated in the workings of the immune system, congenital heart disease, schizophrenia, mental retardation, birth defects, and several cancers including leukemia. But, the scientific team agrees that many more secrets are to be discovered in this decoded text.

Seeing the structure and organization of chromosomes 21 and 22 at the base pair level for the first time immediately suggested new experiments and avenues of research to be pursued. It permits scientists to begin to understand where genes are located on chromosomes, how they express themselves, how deletions that give rise to disease-causing mutations occur, and how chromosomes are duplicated and inherited.

Dunham AR,Hunt JE, Collins R, et al: The DNA sequence of human chromosome 22. Nature, 402(6761):489-95. 1999.

Hattori M, Fujlyama A, Taylor TD, et al: The DNA sequence of human chromosome 21. Nature, 405(6784):311-19. 2000.

Center For Inherited Disease Research: A Service to Help Researchers Identify Genes that Contribute to Human Disease

Background: The Center for Inherited Disease Research (CIDR), located on the Bayview campus of The Johns Hopkins University, provides high-throughput genotyping services, study design advice, sophisticated data warehousing technologies and database assistance to research efforts attempting to identify genetic loci and allelic variants involved in human disease. CIDR is fully funded through a contract from the NIH to The Johns Hopkins University. CIDR is a joint effort by eleven NIH Institutes.

Advance: CIDR has 35 faculty and staff positions encompassing a genotyping lab, statistical genetics, bioinformatics, and technology development. CIDR's application review process began in May 1997, and the first genotyping project commenced in September of that year. Access to CIDR is open to all investigators on a competitive peer review basis. The CIDR Access Committee is a chartered NIH committee that reviews all proposals for scientific quality. A second 5-year contract period (FY2002-FY2006) has been agreed upon.

To date, 58 projects (out of a total of 122 different projects requesting access) have been accepted for genotyping at CIDR, including studies of colon cancer, lung cancer, schizophrenia, Alzheimer's disease, NIDDM, bipolar disorder, non-syndromic deafness, obesity, hereditary nonpolyposis colorectal cancer (HNPCC), osteoporosis, and dystonia. The current genotyping capacity of CIDR is over 3.0 million per year and is projected to increase to 4.8 million genotypes per year in the fall of 2001. CIDR assesses quality using blind duplicate samples provided by the investigators. Beginning with the July 1, 2000 application receipt date, CIDR began accepting applications requesting the genotyping of DNA samples from mice. The genetic markers are linked to the Jackson Laboratory Mouse Genome Informatics database.

Implications: The services provided by CIDR are expediting the identification of genes involved in a variety of diseases. This is a crucial step in understanding the molecular basis of disease and is an early, but vital step in the development of improved diagnostic and treatment strategies.

Brown AS, Feingold E, Broman KW, Sherman SL: Genome-wide variation in recombination in female meiosis: a risk factor for non-disjunction of chromosome 21. Human Molecular Genetics, 9(4):515-23. 2000.

Brzustowicz LM, Hodgkinson KA, Chow EWC, Horner WG, Bassett AS: Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science, 288:678-82. 2000.

Kelley MJ, Jawien W, Ortel TL, Korczak JF: Mutation of *MYH9*, encoding non-muscle myosin heavy chain A, in may-hegglin anomaly. Nature Genetics, 26(1):106-8. 2000.

More information is available at www.cidr.jhmi.edu.

Mammalian Gene Collection: A Resource for Studying Gene Expression and Function

Background: Only about 5% of mammalian genomes contain instructions to make a protein. Sifting through vast sequences in a mammalian genome to identify those functional regions is not a simple task. When a gene is active in a cell, the functional, or coding part of the gene is 'transcribed' into an mRNA molecule as a precursor to making the protein. A particularly powerful material for studying gene expression and gene function, therefore, is cDNA, which represents the full-length, expressed mRNA transcript. Indeed, one of the most effective and widespread manifestations of the genomics revolution has been the ready public access to cDNA libraries, sequences, and clones.

Advance: In FY 1999, numerous NIH Institutes launched an initiative on full-length mammalian cDNAs, the Mammalian Gene Collection (MGC). The project's scientific goals are to produce publicly accessible cDNA clone collections that contain full-length copies of all genes, to sequence these cDNAs, to develop the associated informatics tools, and to create a publicly accessible website to provide up-to-date information to the research community.

In March 1999, a Request For Applications (RFA) was issued to solicit research proposals for improving technology for full-length cDNA production and analysis, and eight awards were made in the fall of 1999. Production activities were begun in earnest during the summer of 2000. cDNA library and EST sequence production are being supported, production cDNA sequencing has just begun, and informatics and data tracking also are part of the project. An External Steering Committee (ESC), comprised of scientists from academia and industry meets regularly by phone and annually in person to oversee and guide the project.

Implications: A major thrust of contemporary biological and biomedical research is to determine and understand the genetic contribution to disease and other biological phenomena. Complete catalogs of genes (both sequences and clones) will be essential for thorough genetic and physiological analysis. The MGC program is designed to generate these critical resources, which will be widely used and of inestimable value to biological researchers.

Strausberg RL, Feingold EA, Klausner RD, Collins FS: The Mammalian Gene Collection. Science, 286(5439):455-7. 1999.

More information can be found on the project's website: http://www.ncbi.nlm.nih.gov/MGC/

Early Childhood Stress Predicts Vulnerability to Alcoholism

Background: Stress is more than an emotion; it is also a physical response that triggers a complex series of biological events in various structures of the brain and in the body. These behavioral and biological patterns define an individual's response to stress. The malleable brains of infants and children are especially affected by long-term or severe social stress, which may result in emotional and biological scars.

Corticotropin-releasing hormone (CRH) is among the hormones that regulate the body's response to stress, and it also contributes to the brain's storage of emotion-related memories. CRH shapes the physical and psychological patterns of coping that the infant brain learns, patterns that can persist into adulthood. Researchers are examining whether high levels of CRH established in response to emotionally disruptive childhood experiences could be an indicator of future vulnerability to alcoholism.

Scientists know that, in rodents, CRH plays a role in establishing the pattern of nervous-system sensitization that occurs in response to stress. For example, when they blocked the effects of CRH in rats' brains by blocking the molecular sites – receptors – to which CRH normally would bind, the rats' response to fear-inducing experiences decreased.

Researchers in this study extended their understanding of CRH's effects in higher species. Like human infants, rhesus monkeys undergo high levels of stress when separated from their mothers. Those that show a vigorous response to this separation develop high levels of stress-related hormones, including CRH, and consume large amounts of alcohol as adults. In monkeys whose parents were absent during infancy, CRH is abnormally high.

Advance: Scientists found that infant monkeys reared apart from their mothers continued to show exaggerated stress reactions to moderately distressful situations as they grew up. At the same time, the levels of CRH in their cerebrospinal fluid (CSF) – the fluid that bathes the brain and spinal cord – were elevated and remained high throughout adulthood. Even in infants reared by their parents, subsequent repeated separations from them resulted in higher CRH levels. When the animals were treated with a compound that blocks CRH receptors, their arousal diminished. Maternally deprived monkeys drank more alcohol, as adults, than did monkeys raised by their mothers. Of several hormones, only CRH correlated with arousal in adult rhesus monkeys.

Implications: These findings indicate that, rather than adapting to repeated stress, CRH receptors become more sensitive to it and that pharmaceutically blocking the receptors may hold therapeutic potential. Parents appear to play an important role in development of the behavioral and physiological response to stress. The new findings suggest that emotionally disruptive early-life experiences that result in anxiety-like behaviors predict alcohol consumption later in life.

Habib KE, Weld KP, Kenner C, et al: Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. Proceedings of the National Academy of Sciences, 97(11):6079-84. 2000.

Alcoholic Fathers' Behavior Predicts Intellectual Deficits in Their Children

Background: Some of the cognitive deficits that appear in heavy drinkers might actually have been present before they began to drink, recent research suggests. These pre-existing deficits may even contribute to heavy drinkers' risk for developing alcohol problems in the first place.

To test this hypothesis, researchers are examining the cognitive functioning of children of alcoholics (COAs), a group known to be at high risk for alcohol problems. Scientists have conducted some studies aimed at determining if these children inherit cognitive deficits that put them at risk for problems with alcohol later in life; however, this line of research has yielded conflicting results. Some studies suggest that COAs have poorer cognitive abilities than do nonCOAs (although data also reveal that these children have IQS well within normal limits), yet others document no difference between children of alcoholic and nonalcoholic families.

The discrepancies in these findings might be due to differences among test subjects. For example, alcoholics with antisocial personality disorder – a disregard for the rights of others that begins in childhood and continues into adulthood – are likely to drink more, begin at an earlier age, and have more alcohol-related problems than do others. Scientists studied elementary-school boys to determine if fathers who have this more severe type of alcoholism are more likely to have sons with learning problems than are non-antisocial alcoholic or nonalcoholic fathers.

Advance: Results of this study support earlier findings that COAs have poorer intellectual functioning than do nonCOAs. Sons of alcoholic fathers with antisocial personality disorder were found to be at highest risk for problems. Researchers also found that differences in academic achievement between COAs and nonCOAs were detectable as early as the first grade. The COAs did not appear to be at risk for severe learning disabilities, but did appear to be on a path to poorer cognitive functioning.

Implications: Behavioral factors, such as stressful family environments, and biologic factors, such as those that occur at various developmental stages, also may account for varying degrees of diminished intellectual ability in COAs. The kinds of deficits found in children of alcoholic fathers with antisocial personality disorder – in verbal processing, abstract planning, and attention ability – are consistent with deficits in frontal lobe function. This area of the brain is believed to play an important role in the development of attention deficit-hyperactivity disorder, conduct disorder, and later problems with alcohol. Regardless of the etiology of the diminished intellectual function of COAs, this study supports the concept that paternal risk characteristics, such as alcoholism and antisocial personality disorder, are early indicators of risk for poor cognitive function in COAs.

Poon E, Ellis DA, Fitzgerald HE, Zucker RA: Intellectual, cognitive, and academic performance among sons of alcoholics, during the early school years: differences related to subtypes of familial alcoholism. Alcohol Clinical and Experimental Research, 24(7):1020-7. 2000.

Children's Emotional Response to Alcohol's Scent on Parents

Background: People's opinions about alcohol form early in life. Studies have shown that, during their first year, infants who have more exposure to parental drinking in their environment react more with pleasure to alcohol-scented toys than do less-exposed infants. Current research suggests, however, that children who are more exposed to alcohol do not necessarily continue to have positive responses to its scent as they grow. Depending on parental drinking habits, just the reverse may be the case. Given the powerful influence that scents exert on emotion, these experiences may affect children's attitudes toward alcohol and, thus, their alcohol-related behaviors later in life.

The olfactory system and the brain region that regulates emotion are connected by a direct neurological link. This unique connection explains why memories evoked by odors are more emotionally charged than those evoked by other sensory systems. Learning studies show that the emotional context in which children sense an odor can influence later behaviors.

For this study, which involved 150 children aged 3.8 to 6 years, investigators determined the prevalence of alcohol use in the home by interviewing all of the mothers and 60 percent of the fathers. In addition to asking about the amount and types of alcoholic beverages consumed, the researchers inquired about the parents' reasons for drinking. The parents' motives for drinking were sought to determine the extent to which they drank to "escape," that is, to change their state of mind, reduce unpleasant moods, or both.

To determine whether the children's reactions to the alcohol scent were related to their parents' drinking habits, the scientists presented the children with a variety of scents, including beer, bubble gum, sour milk, and a neutral odor. The researchers gauged the children's reactions by embedding the test within the context of a game, which made the task age-appropriate and fun, and minimized the impact of language development.

Advance: As expected, regardless of the drinking history of the parents, the majority of children disliked the sour milk odor and liked the bubble gum and neutral odors. The children's reactions to the beer odor, however, varied greatly depending on parental drinking. About half of the children liked the beer odor; of these, almost three-quarters had parents who were *not* escape drinkers. Likewise, of the children who did not like the smell of alcohol, nearly two-thirds had parents who were escape drinkers. The results in these young children concur with reports of aversive learning about alcohol in older, elementary school-aged children of alcoholics.

Implications: It has been argued that expectancies and feelings about alcohol that develop during early childhood may influence alcohol use in adolescence. This study suggests that at least some early learning about alcohol is based on sensory experiences and the emotional context of parental drinking. A question for future research is whether these early emotional responses to the scent of alcohol persist and can explain later behaviors.

Mennella JA, Garcia Pl: Children's hedonic response to the smell of alcohol: effects of parental drinking habits. Alcohol Clinical and Experimental Research, 24(8):1167-71. 2000.

Understanding the Immune System of HIV-Infected Adolescents

Background: T lymphocytes ("T-cells"), a type of white blood cell, grow and mature in the thymus gland, an organ of the lymphatic system located in the upper region of the chest. *Naïve* T-cells protect the body from new infections, while *memory* T-cells protect the body from pathogens it has previously encountered. As individuals age, the function of the thymus gland decreases and the production of naive cells is reduced. Some researchers believe that the thymus gland stops functioning in childhood and, after this point, mainly memory T-cells remain. However, recent work has demonstrated that certain naive T-cells circulating in the bloodstream can be recovered after T-cells are damaged from bone marrow transplantation or chemotherapy, but usually only in children and young adults. To address the lack of information about the *adolescent* immune system, scientists conducted a study to understand the immune response of both HIV-positive and HIV-negative teens.

Advance: Researchers found that HIV-positive youth, many of whom were infected relatively recently, have a significant increase in certain naive T-cells compared to HIV-negative youth, a unique finding in this population. Researchers also noted that HIV positive youth had a significant increase in certain memory T-cells compared to HIV-negative youth.

Implications: The presence of high levels of these naive cells suggests that there is functioning thymic tissue in some adolescents infected with HIV. This indicates that the immune system of HIV-infected adolescents may be capable of better responding to new infections, including HIV, than the immune system of infected children or adults. Therefore, HIV-infected adolescents may have an immune system that is capable of being restored following intensive anti-viral therapy. With HIV-infection rates continuing to rise for adolescents, this finding has important implications for successfully treating this increasingly at-risk population.

Douglas SD, Rudy B, Muenz L, et al: T-lymphocyte subsets in HIV-infected and high-risk HIV uninfected adolescents: retention of naive t-lymphocytes in HIV-infected adolescents. Archives of Pediatrics and Adolescent Medicine, 154(4):375-80. 2000.

"Nerve Sprouting" May Be Useful Target for Preventing Heart Rhythm Disturbances

Background: Approximately 250,000 Americans die each year from sudden cardiac death caused by unexpected abnormal heart rhythms (arrhythmias). Most of these victims have long-standing heart damage from previous heart attacks or heart failure. The arrhythmias are usually triggered by increased activity of the nervous system. It has long been known that the nervous system affects the heart's rhythm and pumping efficiency. Impulses from one branch of the nervous system increase both the rate at which the heart beats and the force with which it pumps, while impulses from another branch of the nervous system slow the heart and reduce the strength of each heart beat. Researchers are studying nervous system involvement in heart rhythm disturbances in order to develop strategies for preventing them.

Advance: Recent studies show that new excitatory nerves grow into areas of the human heart severely damaged by a heart attack or by development of heart failure. This "sprouting" of new nerves occurs only near the edge of the damaged heart region, where it increases the likelihood of life-threatening arrhythmias. In parallel studies on dogs with chronic heart damage, researchers showed that a similar pattern of artificially induced nerve regrowth is accompanied by arrhythmias leading to sudden cardiac death.

Implications: These findings suggest a potential target – the biological events involved in "nerve sprouting"– for treating and preventing abnormal heart rhythms. Strategies targeting "nerve sprouting" might not only prevent sudden cardiac death in many individuals, but also provide more effective, less costly treatment for the thousands of patients who survive life-threatening arrhythmias and now rely on treatments such as implantable defibrillators to shock their hearts electrically out of dangerous rhythms. [secondary – treatment]

Cao JM, Fishbein MC, Han JB, et al: Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. Circulation, 101(16):1960-9. 2000.

Cao JM, Chen LS, KenKnight BH, et al: Nerve sprouting and sudden cardiac death. Circulation Research, 86(7):816-21. 2000.

Maternal Immune Tolerance of the Fetus

Background: Implantation establishes the connection between the mammalian embryo and the mother. During implantation, the embryo and placenta invade the mother's uterus and establish a connection with the maternal blood supply. How the fetus is protected from being rejected by the maternal immune system remains a critical question.

Advance: Scientists have identified a unique cell-surface molecule on the rhesus monkey placenta that shares many characteristics with a human placental molecule, HLA-G. The researchers have demonstrated that the rhesus molecule – a major histocompatability (MHC) class I molecule known as Mamu-AG – is expressed at the critical stages when the embryo/placental complex is connecting to the maternal blood supply within the uterus. Mamu-AG expression is believed to prevent the maternal immune cells from recognizing the embryo as foreign and rejecting it.

Implications: The monkey is an invaluable model for examining the molecular and immunological factors that contribute to the establishment and maintenance of pregnancy, studies which cannot be done in humans. The model allows research to be conducted at the very earliest stages of pregnancy, a time in which the greatest number of embryonic losses occurs in humans. The findings suggest that inadequate expression of this type of MHC Class I molecule might substantially impair the invasiveness, survival, and function of the embryo/placental complex; alter the blood flow between the uterus and placenta; and increase the risk for development of pre-eclampsia, a complication of pregnancy marked by high blood pressure and fluid retention.

Slukvin II, Lunn DP, Watkins DI, Golos TG: Placental expression of the nonclassical MHC class I molecule Mamu-AG at implantation in the rhesus monkey. Proceedings of the National Academy of Sciences, 97(16):9104-9. 2000.

Mutations Within a Skeletal Muscle Gene Cause Genetic Muscle Disease

Background: Electrical charges exist both inside and outside of all cells. The difference between these two charges constitutes the voltage potential, maintained by the transfer of sodium, potassium, chloride and other charged ions through specialized channels in the cell membrane. The ability of a muscle to contract depends upon the state of this potential within its constituent cells. Myotonia congenita (MC) is a genetic disease associated with abnormalities in the chloride channel of skeletal muscle manifested by intermittent and progressive weakness. To date, more than 35 mutations, or variants, of the chloride channel gene (CLCN1), located on chromosome 7q35, have been identified. Patients afflicted with MC have clinical symptoms of varying severity.
Advance: Investigators at the University of Utah General Clinical Research Center have characterized the physiological effects of five such mutant genes by isolating them and then introducing them into cells growing in culture. Studies on these cells documented the altered ability of chloride ions to pass through each of these uniquely modified channels, thereby simulating characteristics of the disease variants.

Implications: The ability to ascribe clinical symptoms of rare, genetic diseases to the defined alterations of proteins produced by mutated genes will allow pharmaceutical researchers to design drugs specific for these afflictions in a more timely and cost-effective manner.

Zhang J, Bendahhou S, Sanguinetti MC, Ptácek LJ: Functional consequences of chloride channel gene (CLCN1) mutations causing myotonia congenita. Neurology, 54(4):937-42. 2000.

The Assembly of Neural Circuits

Background: How precisely connected circuits of nerve cells form during development is one of the great mysteries of biology, a puzzle that has profound implications for understanding and treating many disorders. The formation of connections depends on a series of steps that includes the extension of axons (nerve fibers) toward distant targets, the selection of target regions by growing axons, and the formation of selective synapses (functional connections). There is increasing evidence that transcription factors – signal molecules that turn genes on and off – control many aspects of neural development. However, to date, transcription factors have been implicated in regulating very early, global events in development such as specification of the overall shape and pattern of brain structure, determination of specific nerve cell types, and selection of the early paths that axons grow, rather than in specifying the detailed connections among billions of nerve cells.

Advance: Scientists have now discovered a transcription factor that appears to control the formation of specific connections in spinal cord circuits. They studied the connection between sensory neurons, which grow into the spinal cord, and motor neurons, which lie within the cord. The research team first showed that the transcription factor Er81 is present in both the sensory and motor neurons at the critical time when the connections are formed. This implies that ER81 might regulate a gene that is important for the formation of synapses. Subsequent experiments demonstrated that mice with a mutant, inactive form of Er81 are severely uncoordinated because they fail to form the sensory to motor connection properly, despite the presence of normal sensory and motor neurons.

Implications: These findings are an important step in unraveling the signals that control the formation of nerve cell circuits. Understanding that process is important for progress against the many developmental disorders in which the nervous system becomes wired up improperly, including some forms of epilepsy and perhaps autism, to name just two. Finding ways to stimulate the formation of appropriate connections is also becoming the main limiting factor for regeneration and functional recovery in the adult brain and spinal cord, now that there has been significant progress in coaxing axons to regrow after damage. As the signals that control the initial wiring up of the nervous system become clear, they may be useful in regeneration therapies that attempt to recapitulate steps in the development process. A great deal of fundamental research is necessary before these applications can become reality.

Arber S, Ladle DR, Lin JH, Frank E, Jessel TM: ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. Cell, 101(5):485-98. 2000.

Sex Hormones Provide Clues to Development of Autoimmunity

Background: Female sex is a known contributor to increased risk for autoimmune diseases such as lupus, rheumatoid arthritis, and multiple sclerosis. In these diseases, the immune system becomes impaired, and immune responses against the body's own tissues develop.

Advance: Working with an animal model genetically manipulated to produce antibodies that react against the animal's own tissue, investigators looked at the effects of the female hormone estradiol on the initiation of immune responses directed against self tissue. They noted that estradiol makes some immune cells resistant to signals that produce tolerance, a state of nonresponsiveness to substances that could otherwise cause disease. In the genetically manipulated mice, administration of estradiol brought on lupus-like disease, whereas in normal mice estradiol had no effect on cells.

Implications: Knowledge about the interactions between sex hormones and the development of autoimmunity will improve understanding of the mechanisms of disease.

Byone M, Grimaldi CM, Diamond B: Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. Proceedings of the National Academy of Sciences, 97(6):2703-8. 2000.

Extent of Viral Diversity Early in Hepatitis C Virus Infection Predicts Whether the Infection will Become Chronic

Background: Approximately 80% of persons infected with the hepatitis C virus (HCV) become persistent carriers of this virus and generally have associated evidence of chronic hepatitis. In approximately 20%, the chronic hepatitis may progress to cirrhosis and liver-related fatality. The reason why most individuals fail to clear the virus is unknown and there has been no way to predict who will recover from HCV infection and who will develop persistent infection.

Advance: Using stored samples previously obtained in prospective studies of transfusion-associated hepatitis C, scientists extracted and amplified viral nucleic acid (i.e., "genetic material") at various time points early in HCV infection and then measured changes (mutations) in the nucleic acid structure of the viral envelope. It had been known that HCV exists as a population of closely related but immunologically distinct variants that have been termed the quasispecies. By cloning and sequencing, investigators determined the number of viral variants and the extent to which they differed (diversity) at each of four time points during the first 16 weeks of infection. It was shown that in patients who recovered from HCV infection, the degree of viral diversity decreased as the patient developed antibodies to HCV. This decrease in diversity indicated that the immune response was able to contain viral replication and in each case was followed by clearance of the virus and recovery from HCV infection. In contrast, patients who went on to develop chronic infection showed a dramatic increase in viral diversity in response to antibody production by the host. In these cases, the immune response was insufficient to contain all the viral variants and those that escaped the immune response continued to replicate and resulted in persistent infection.

Implications: These findings add to our knowledge of the natural history of HCV infection and demonstrate that early in the infection a battle is waged between the virus and host immune responses. While this host-virus interplay is typical of most viral infections, HCV is novel in that it exists in multiple immunologically distinct forms (quasispecies). Thus to be effective, the antibodies that are generated by the host need to neutralize all or most of the HCV variants. If the antibodies do not achieve this broad neutralization, they drive the most divergent, immunologically resistant species to replicate at higher level resulting in persistent infection. This implies that HCV infection can be fought not only with anti-viral drugs, but also with therapeutic approaches that would broaden and intensify the immune response. Such immune enhancement would be ideally applied early in HCV infection, but would also be applicable in those with established chronic infection.

Farci P, Shimoda A, Coiana A, et al: The outcome of acute heptitis C predicted by the evolution of the viral quasispecies. Science, 288(54640:339-44. 2000.

Animal Model Shows Pain and Tissue Injury in Newborns Alters Nerve Circuitry and Reaction to Pain Later in Life

Background: Newborns who experience tissue injury and pain during critical periods of development may undergo a permanent rewiring of their nervous system that increases their sensitivity to pain later in life. Each year, more than 400,000 babies in the U.S. are born either prematurely or at a low birth weight. Not only are these babies confronted with the trauma of living in the outside world too soon, but available medical procedures used to keep them alive and monitor their progress may cause pain and tissue injury. Heel sticks to draw blood, the insertion of IV lines, and the use of ventilators are some of the modern technologies that are both miraculous and difficult. There has been considerable debate over the existence of pain in newborns and its management.

Advance: Working with an animal model, scientists at the NIH have provided the first physical evidence that pain and inflammation in newborns alters the development of pain pathway circuitry, causing a stronger response to pain in adulthood. The study used newborn rat pups to explore the effect of tissue injury and pain on the development of pain pathways. An irritant was injected into the left hind paw of 1-day-old pups, an age equivalent to 24 weeks gestation in humans. When the animals were examined as adults, it was found that they had an increase in the density of nerve fibers on the left side of the dorsal horn, the layered structure in the spinal cord that propels brain signals up to the brain. They also reacted more strongly to pain as adults. However, when rats received the left hind paw injection on postnatal day 14, an age equivalent to adolescence in humans, the patterns of nerve fibers looked like those of normal rats. The researchers surmise that the critical time point responsible for a change in input had passed by day 14.

Implications: The changes that occur because of tissue injury and pain are likely not limited to the spinal cord, but also may involve higher centers of the brain that are part of pain pathways. The study suggests that pain is an important early birth stimulus that may have a lifelong impact, and that it is essential to develop approaches to limit or prevent its effects.

Ruda MA, Ling QD, Hohmann AG, Peng YB, Tachibana T: Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science, 289(5479):628-30. 2000.

Patterned Entry by Epstein-Barr virus in Polarized CR2-Positive Epithelial Cells

Background: Epstein Barr virus (EBV), a gamma herpes virus has been identified as an important disease producing organism in the patient whose immune system has been compromised. This paper describes how EBV, must cross the epithelium prior to viral entry. Previous papers dealing with the acute EBV infection have not addressed this unique property of epithelial cells, their intrinsic polarity or sidedness. In polarized epithelium the direction of viral entry and release correlates with the tendency of a virus to establish local versus whole body infection. Unequal distribution of proteins and fats in the cell membrane along with junctions between adjacent cells creates areas, one facing the external environment and the other contacting the internal cells and tissues. This sidedness has implications for pathogenesis since basolateral (internal) infection of the virus provides more opportunity for systemic spread of the virus and interaction with the immune system.

Advance: EBV, whose principal tissue reservoir is immune system B-lymphocytes, also has disease manifestations in epithelium, such as nasopharyngeal carcinoma, oral hairy leukoplakia suggesting inter-tissue spread perhaps influenced by epithelial cell polarity.

Implications: These data should provide insight into the molecular events used by EBV to gain entry into cells. Understanding these events should lead to the design of new therapeutic modalities.

Chodosh J, Gan YJ, Holder VP, Sixbey JW: Patterned entry by epstein-barr virus in polarized CR2-positive epithelial cells. Virology, 266(2):387-96. (2000).

Regulation of Nematode Life Span Through Sensory Perception

Background: It is now clear that aging can be altered, and even extended, by genetic interventions. The full range of this extension is currently a very intense research area.

Advance: Mutations that cause defects in the sensory perception pathways of the nematode *C. elegans* extend the life span of the organism. These sensory pathways respond to temperature, substances that the nematode can taste or smell, and mechanical stimuli. Although the mechanism of life span extension is not known, it could indicate that this nematode is able to slow down aging in response to starvation conditions, thus living longer to ensure that it will eventually have a chance to produce its full quota of offspring. This may be comparable to the well-documented extension of mammalian life span by caloric restriction, thereby providing a model system for studying the mammalian caloric restriction paradigm.

Implications: The elucidation of ways to extend the life span of model systems provides information that may prove useful for developing human interventions to slow deleterious age-related changes. This study also provides a new model system for studying signal transduction mechanisms with implications for understanding aging.

Apfeld J, Kenyon C: Regulation of lifespan by sensory perception in *Caenorhabditis elegans.* Nature, 402(6763): 804-9. 1999.

Enteral Feeding Options Influence Corticosterone Patterns in Rats

Background: With enteral nutrition, nutrients are delivered by way of a tube placed directly into to the stomach or small intestine. This intervention is being prescribed at an unprecedented rate in both the hospital and home. From 1989 to 1992 the number of Medicare patients on home enteral feedings doubled. There are many enteral feeding options using different formula ingredients, fiber levels, delivery methods and feeding schedules. It is not clear which enteral nutrition delivery and composition options are most physiologically appropriate.

Time patterns exist in biological systems and synchronization of patterns across biological systems is thought to be necessary for health and well-being. Glucocorticoids that are essential for metabolism and fighting infection have a 24-hour temporal pattern that may be altered with certain feeding schedules. The purpose of this study was to characterize corticosterone temporal patterns while systematically varying 16 enteral feeding options in a well-established nutritional animal model.

Advance: Only 5 of the 16 feeding schedules resulted in a more normal 24-hour rhythmic pattern of corticosterone release. Using a 24-hour feeding schedule, rather than a 12 hour schedule, resulted in a more normal pattern of corticosterone release no matter whether the feeding was given continuously or as a bolus (3.33 kcal per hour continuously or 10 kcal every 3 hours over 24 hours versus 6.67 kcal per hour continuously or 26.66 kcal every 4 hours over 12 hours). Inclusion of fiber in the feeding formula resulted in a more uniform rhythm of corticosterone release. Restricted caloric intake delivered on a bolus schedule resulted in the highest midline estimate of rhythm of corticosterone.

Implications: Direct extrapolations from rats to humans cannot be made. However, this investigation suggests that enteral feeding schedules and fiber content are important factors in avoiding disruption of the temporal pattern of corticosterone release with its implications for cellular metabolism and the stress response.

Westfall UE: How enteral feeding options influence corticosterone patterns in rats. Biological Research for Nursing, 1(3):233-44. 2000.

Effect of Female Gonadal Steroids on Stroke Injury

Background: Disease of the blood vessels in the brain is the third leading cause of death in the United States. Total costs for stroke are estimated to be $43 billion/year, of which the indirect portion from lost productivity and other factors estimated at about $15 billion/year. The most important risk factors for stroke are hypertension, heart disease, diabetes, and cigarette smoking. Others include heavy alcohol consumption, high blood cholesterol levels, illicit drug use, and genetic or congenital conditions, particularly vascular abnormalities.

A growing area of investigation is the influence of estrogen on risk and outcomes of cerebrovascular injury in women. A series of studies have investigated the effect of estrogen and progesterone on brain injury in an animal model.

Advance: In female mice, blocking of cells in blood vessels that receive messages from estrogen worsened damage from stroke. This finding suggested that estrogen inhibited stroke injury by mechanisms not linked to preservation of blood flow in the brain. Similarly, blocking the intracellular estrogen receptor subtype-a did not worsen tissue damage, suggesting that estrogen inhibited brain injury by mechanisms independent of this receptor type. Unlike estrogen which the study demonstrated to have protective effects, chronic progesterone therapy exacerbated stroke injury in female rats.

Implications: These findings need to be tested in humans. If they are found to be true, there are tremendous implications in terms of understanding the effects of sex hormones on the mechanisms and outcomes of stroke injury. An area of particular interest is use of hormone replacement therapy in postmenopausal women since it is estimated that estrogen and progesterone alone and in combination are used to treat many millions of women in the United States.

Sawada M, Alkayed NJ, Goto S, et al: Estrogen receptor antagonist ICI182,780 exacerbates ischemic injury in female mouse. Journal of Cerebral Blood Flow and Metabolism, 20:112-18. 2000.

Sampei K, Goto S, Alkayed NJ, et al: Stroke in estrogen receptor-a-deficient mice. Stroke, 31(3):738-44. 2000.

Murphy SJ, Traystman RJ, Hurn PD. Progesterone exacerbates striatal stroke injuiry in progesterone-deficient female animals. Stroke, 31(5):1173-8. 2000.

Malaria Parasite Development in a Fruit Fly Model

Background: Malaria kills more than 1 million persons annually, and almost half of the world's population lives in areas where they are at risk of contracting the disease. Parasites from the genus *Plasmodium* cause malaria and, each year, about 110 million people develop the disease. *Plasmodium parasites* are transmitted to humans by the *Anopheles* mosquito. The complex *Plasmodium* life cycle, consisting of multiple stages of development, takes place in both humans and in the mosquito. When a female *Anopheles* mosquito bites a person who already is infected by malaria, the mosquito ingests blood that contains malaria parasite. In the mosquito, malaria parasites complete a number of developmental steps, including several developmental changes and many rounds of nuclear division. During these processes, the malaria parasites move from the mosquito's digestive tract to its salivary glands. In the salivary glands, the parasites are in the form of infectious sporozoites that can be released into the blood of another person when the mosquito bites again. How malaria parasites survive in the mosquito and receive signals to change from one form to another is not known. Because the *Anopheles* mosquito cannot be easily managed within the laboratory environment, its biochemical and genetic makeup is not well defined, and little is known about the response of the mosquito's immune system to infection by the malaria parasite.

Advance: Scientists have now learned how to grow *Plasmodia* in the fruit fly (*Drosophila melanogaster)*. Mosquito and fruit fly physiology, including the functions of their immune systems, are similar because the insects are closely related. Moreover, the fruit fly has a long history of laboratory use and the *D. melanogaster* genome has been fully sequenced. The ability to cultivate *Plasmodia* in an insect whose genome sequence is fully known allows investigators to identify the specific genes involved in the fruit fly's response to *Plasmodium*, and then to look for the corresponding genes in the mosquito. Thus, the fruit fly provides an excellent model for studying the details of these insects' immune-system responses to infection by *Plasmodium*. After successfully studying the development of infectious sporozoites in *D. melanogaster*, scientists determined that macrophage-like cells (cells in the insect that are equivalent to the specialized human white blood cells that engulf and destroy microorganisms) play a key role in the fruit fly's immune defenses. In addition to shedding light on probable mechanisms of the *Anopheles* mosquito's immune-system response to *Plasmodial* infection, this discovery demonstrates the usefulness of using *D. melanogaster* as a model to study the insect immune response to *Plasmodial* infection.

Implications: Use of *D. melanogaster* as a model should significantly speed the pace of malaria research, accelerating efforts to understand the response of the mosquito's immune system to malaria and to determine factors critical to malaria transmission. In turn, that knowledge may accelerate the discovery and development transmission-blocking vaccines.

Schneider D, Shahabuddin M: Malaria parasite development in a *Drosophila* model. Science, 288(5475):2376-79. 2000.

Alcohol Raises Risk of Brain Damage in Addicted Adolescents

Background: Adults who go through repeated bouts of heavy alcohol use and withdrawal run the risk of changes in brain functions, like memory and learning, studies show. Now, researchers are asking if children who follow the same drinking pattern also risk brain damage.

The human brain continues to develop during adolescence. On one hand, repeated heavy alcohol use and withdrawal could interfere with biologic events that occur during adolescent brain development, which could lead to unique kinds of brain damage. On the other hand, the development that occurs during adolescence could provide enough resilience to compensate for the damage that alcohol would cause in an adult brain. The reality could lie somewhere in between, with the developmental changes of adolescence contributing to greater brain damage in alcohol-dependent teens in some respects and protecting them from alcohol-related brain damage in others.

To examine this spectrum of possibilities, researchers tested a group of 15- to 16-year old alcohol-dependent children who also used, but were not dependent on, other substances. All reported having used alcohol at least 100 times, heavily at least once in the previous 3 months. After the teens had been abstinent for 3 weeks, the researchers tested their learning, memory, language skills, problem-solving abilities, and attention. They compared the results with those of a control group of children of similar age, gender, socioeconomic status, and education who had never abused substances.

Advance: The scientists found differences between the two groups in memory function and in the ability to comprehend and conceptualize pictures and other visual cues (visuospatial cognitive function). Adolescents with a history of long-time heavy drinking had more difficulty remembering and recalling verbal and nonverbal information. Children who reported having recently experienced (but prior to the three-week abstinence period) alcohol withdrawal symptoms were more likely to have poorer visuospatial function. Compared to others, children reporting several bouts of withdrawal over their lifetime had a harder time retrieving verbal and nonverbal information.

Implications: This preliminary work offers compelling evidence that a pattern of heavy drinking and withdrawal puts adolescents at risk for brain damage. Researchers also must ask whether the neuropsychological deficits seen in these adolescents were present before they began drinking, perhaps predisposing them to, and compounding, their problems with alcohol. These kinds of studies lead to methods of identifying teens at risk and optimal biologic and behavioral points for intervention.

Brown SA, Tapert SF, Granholm E, Delis DC: Neurocognitive functioning of adolescents: effects of protracted alcohol use. Alcohol Clinical and Experimental Research, 24(2):164-71. 2000.

Smoking and Alcoholism: A Genetic Link?

Background: Smokers drink and drinkers smoke. The cliché of the smoke-filled party with an ash tray next to every drink is supported by statistics: people who consume alcohol are more likely than nondrinkers to smoke cigarettes, and they smoke more heavily than the average smoker. A survey of alcoholics in general hospitals revealed that approximately 85 percent were smokers. Most people addicted to alcohol also are addicted to nicotine and are less successful than nonalcoholics at stopping smoking.

The combined use of tobacco and alcohol is a public health concern. Tobacco use alone causes more than 400,000 deaths per year in the United States, and tobacco-related conditions are associated with 36 percent of all deaths. Chronic alcoholics often survive alcohol-related disorders only to die from illnesses caused by smoking.

The role of genes in contributing to vulnerability to alcoholism is well established, and considerable evidence reveals that genetic factors are responsible for about 50 percent of a person's risk of becoming addicted to smoking. Given these phenomena, a logical research question to ask is if some shared genetic factor is at least partly responsible for this dual addiction.

Scientists asked 3,400 pairs of middle-age male twins to describe their drinking and smoking habits. Participants were divided into two groups: identical twins, who share exactly the same genetic inheritance, and nonidentical twins, who are no more alike than any randomly selected pair of brothers. The patterns of inheritance of smoking and drinking behaviors between these two groups were compared statistically.

Advance: Analysis revealed that the tendency for alcoholism and nicotine addiction to occur together in the same person is largely due to genetics. Shared family-environment influences appeared to play only a minor role. These results suggest that nicotine addiction among alcoholics is not merely a coincidence or a cultural stereotype, but part of the larger risk for becoming addicted to both substances.

Implications: These results have implications for both the prevention and treatment of alcohol and tobacco addiction. Adolescents experimenting with tobacco and alcohol have little understanding of the powerful role genes play in determining their risk for becoming addicted to both drugs. This should be stressed in efforts to prevent teenage smoking and drinking. The dual nature of smoking and drinking heritability also supports the practice of combining alcoholism treatment with a smoking cessation program, which has not been traditional practice.

True WR, Xian H, Scherrer JF, et al: Common genetic vulnerability for nicotine and alcohol dependence in men. Archives of General Psychiatry, 56(7):655-61. 1999.

Craving for One Drug May Increase Cravings for Other Drugs

Background: It is very common for individuals to abuse more than one drug. For example, research has shown that more than two-thirds of drug abusers also smoke cigarettes on a regular basis. Of those who attempt to quit smoking, they report that the hardest part is fighting the intense craving for the drug, in this case nicotine, during abstinence. Craving is thought to be caused, in part, by environmental cues associated with drug using behaviors. The biological and psychological mechanisms of craving are yet to be thoroughly understood by the scientific community. One approach that is showing great promise is the ability to compare smokers' responses when exposed to neutral and smoking related cues in a laboratory setting.

Advance: New findings suggest that craving for nicotine appears to increase craving for other illicit drugs among drug abusers who smoke tobacco. A recent study examined the relationship between craving for nicotine and other drugs, using a technique of "cue-induced" craving. Participants listened to recorded scripts describing scenes that were pleasant, unpleasant, or neutral. Some of the scripts mentioned smoking while others did not. The participants reported that their tobacco craving increased in intensity when listening to the smoking messages in the scripts. They also reported that craving for tobacco also elicited craving for the participant's other drug of choice.

Implications: These results suggest that smokers in drug treatment programs may be less successful than nonsmokers in staying off drugs. Therefore, to be more successful drug treatment programs should offer smoking cessation programs to individuals addicted to nicotine.

Taylor RC, Harris NA, Singleton EG, Moolchan ET, Heishman SJ: Tobacco craving: intensity-related effects of imagery scripts in drug abusers. Experimental and Clinical Psychopharmacology, 8(1):75-87. 2000.

Long-Term Behavioral Effects of Iron Deficiency Anemia in Infancy

Background: Iron deficiency in infancy is a common problem which causes altered behavior and development. Approximately 20 to 25 percent of all infants in the world have iron deficiency anemia, and many more have iron deficiency without anemia. In the United States, poor and minority children are at increased risk. Overall, five percent of poor African American and Hispanic infants and toddlers, and specifically 18 percent of poor Mexican American infants have iron deficiency anemia.

Advance: NIH-supported investigators evaluated cognitive, socioemotional, motor skills and school functioning of a group of Costa Rican children between 11 and 14 years of age who had tested positive and had been treated for iron deficiency anemia as infants. Researchers compared the outcomes with a matched group of children who had good iron status in infancy. Both groups of adolescent children were free of iron deficiency and growing normally by U.S. standards; however, the investigators found that those children who had severe, chronic iron deficiency in infancy scored lower on measures of mental and motor function when tested in adolescence. Significant differences were found in arithmetic achievement, written expression, motor functioning, spatial memory, and selective recall. Twice as many of the adolescents who had been anemic infants had repeated a school grade compared to members of the nonanemic cohort and three times as many required special services. Parents and teachers rated the behavior of the formerly anemic adolescents as more problematic, especially related to anxiety/depression, social and attention problems.

Implications: Severe, chronic iron deficiency anemia in infancy has permanent effects on brain function. This nutritional anemia should be diagnosed and treated as early as possible to ensure normal brain development and function throughout life. Early screening for iron deficiency anemia is particularly important for minority infants, because of their increased risk for the condition, especially infants who are African American, Hispanic, and more specifically Mexican American.

Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW: Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics, 105(4):1-11. 2000.

Treatment of Trichomoniasis Increased the Risk of Preterm Birth

Background: Trichomoniasis, a common genital tract infection caused by the protozoan organism, *Trichomonas vaginalis*, has been associated with an increased risk of preterm birth. Since most women with this infection do not have symptoms, some experts have recommended that pregnant women be routinely screened for its presence, and women who have the infection be treated. However, there has never been a randomized clinical trial to determine whether treatment of trichomoniasis will prevent preterm birth. Currently, women are not routinely screened for this condition and thus, do not undergo treatment.

Advance: NIH-supported researchers screened pregnant women for the presence of trichomoniasis during the second trimester of pregnancy. Women who were infected with trichomoniasis were divided randomly into two groups to receive either the drug metronidazole (the only effective treatment for this condition), or identical-appearing placebo capsules. The researchers then compared the occurrence of premature birth in the treated group with that in the control group. The study was stopped ahead of schedule by an external monitoring board when, unexpectedly, the occurrence of premature birth was significantly higher in the women who received metronidazole. Premature birth occurred to 19 percent of women assigned to be treated and to only 10.7 percent of women assigned to receive the placebo treatment. The difference was entirely attributable to an increase in spontaneous premature labor among women receiving metronidazole. These differences occurred despite the fact that metronidazole was 93 percent effective at eliminating trichomoniasis.

Implications: In addition to providing evidence against the value of screening of pregnant women without symptoms for trichomoniasis, this study raises serious concerns about the wisdom of using of antibiotics indiscriminately, particularly metronidazole, during pregnancy. If treatment of an infection during pregnancy might increase the occurrence of premature delivery, then researchers and clinicians must be extremely cautious not to recommend screening and treatment of asymptomatic infections until the benefits of this practice have been demonstrated in well-designed research studies.

Carey JC, Klebanoff MA, Hauth JC, et al: Metronidazole to prevent preterm dilivery in pregnant women with asymptomatic bacterial vaginosis. National institute of child health and human development network of maternal-fetal medicine units. The New England Journal of Medicine, 382(8):534-40. 2000.

Dietary Sodium Intake Increases Risk of Cardiovascular Disease in Overweight Adults

Background: Observational studies have repeatedly identified an independent, positive relationship between dietary intake of sodium and blood pressure level. Moreover, clinical trials have demonstrated that reduced sodium intake leads to a reduction of blood pressure in both patients with hypertension and persons with normal blood pressure. Although high blood pressure is strongly associated with risk of stroke and heart attack, the link between a high-sodium diet and these adverse events has not been clearly established.

Advance: Researchers following a large, representative group of U.S. adults for 20 years found that high sodium intake is strongly associated with an increase risk of mortality, particularly cardiovascular disease mortality, in overweight persons. Among adults who were overweight, those who consumed the highest amounts of sodium were 63 percent more likely to die of cardiovascular disease than those who consumed the lowest amounts of sodium. No significant association between dietary sodium intake and risk of cardiovascular disease was found in normal-weight persons.

Implications: Average sodium intakes in the adult U.S. population are well above the recommended level of 2,400 mg. per day. Moreover, overweight is an important, increasingly common risk factor for cardiovascular disease. These findings suggest that interventions to prevent cardiovascular disease should recommend both weight loss and sodium reduction and that, among persons who have difficulty losing weight, greater attention to reductions in salt intake may be warranted.

He J, Ogden LG, Vupputuri S, et al: Dietary sodium intake and subsequent risk of cardiovascular disease in overweight adults. Journal of the American Medical Association, 282(21):2027-34. 1999.

Improving Management of Asymptomatic Hyperparathyroidism

Background: Hyperparathyroidism (HPT) affects 100,000 Americans each year; women outnumber men by two to one, and the risk increases with age. The disorder is caused by one or more enlarged, overactive parathyroid gland(s) which produce too much parathyroid hormone (PTH). PTH helps maintain the correct balance of calcium and phosphorous in the body by regulating the release of calcium from bone, absorption of calcium in the intestine, and excretion of calcium in the urine. With excessive PTH, blood calcium levels rise, a condition referred to as hypercalcemia. In part, this increased blood calcium is due to transfer of calcium from the bones to the blood, which may weaken the bones and increase risk for fractures. Calcium levels may also increase in the urine, causing kidney stones. Although HPT can cause severe bone and kidney disease, it is increasingly being diagnosed in patients who have few or no symptoms, as a result of routine measurement of blood calcium levels. Currently, surgery to remove the enlarged gland is the only treatment for this disorder. In 1990, a panel of experts convened by the NIH concluded that asymptomatic patients with mild disease may not need immediate treatment, and that close monitoring for complications of the disorder was an alternative to surgery. They recommended research to assess long-term outcomes of medical follow-up and risk of progression of complications in these patients.

Advance: Researchers have now demonstrated that surgical removal of the affected parathyroid gland(s) can provide significant benefits for patients with asymptomatic or mild HPT. Based on the NIH Consensus Panel's recommendations, researchers studied the clinical course of 121 patients with primary HPT, 101 of whom were asymptomatic. Over a period of ten years, most of the 52 patients with asymptomatic HPT who did not undergo surgery remained stable. None developed kidney stones, loss of kidney function, fractures, or life-threatening hypercalcemia. However, 14 (27 percent) of these patients did have clinical progression, as defined by increases in blood or urine calcium levels or decreased bone density. While bone density was generally stable in the asymptomatic patients who did not undergo surgery, the asymptomatic patients who did have surgery generally had a substantial and sustained increases in bone density. Bone density improved both in the spine and the femoral neck, where hip fractures tend to occur, but not in the wrist.

Implications: These results show that many patients with mild or asymptomatic HPT will have a significant, rapid and sustained improvement in bone density and other benefits from surgery. The study also provides information on which patients are most likely to benefit from surgery. Since the 1990 panel made its recommendations, there have been important developments that have made surgery for HPT safer, simpler and faster. Now, abnormal glands may be identified prior to surgery using advanced imaging technologies. Also, rapid measurement of PTH during surgery provides timely information on whether the abnormal gland(s) have been successfully removed. These new technologies can improve the success of surgery in curing the disorder.

Silverberg SJ, Shane E, Jacobs TP, Siris E, Bilezikian JP: A 10-year prospective study of primary hyperparathyroidism with or without parathyroid surgery. The New England Journal of Medicine, 341(17):1249-55. 1999.

Prolonged Beneficial Effect of Intensive Blood Glucose Control on the Complications of Diabetes

Background: The Diabetes Control and Complications Trial (DCCT), conducted from 1983 to 1993, was a multicenter study of over 1,400 people with type 1 diabetes. The goal of this trial was to compare conventional therapy with intensive therapy to maintain blood glucose and glycosylated hemoglobin levels as close to normal as possible in order to determine whether intensive therapy affects the development of eye, kidney, and nerve complications associated with diabetes. Glycosylated hemoglobin measures blood glucose levels over a two-three-month period, providing more information about overall control of blood glucose than individual measurements. The DCCT demonstrated that the intensive therapy group had greatly reduced development of diabetic eye, kidney and nerve disease when compared to the group who had received conventional therapy. All patients received subsequent care from their individual physicians. Virtually all DCCT participants are being followed in the "Epidemiology of Diabetes Interventions and Complications" (EDIC) study, an observational examination of the later-term complications of diabetes.

Advance: In the DCCT, glycosylated hemoglobin was significantly lower in the intensive therapy group compared with the conventional therapy group. This difference resulted in a marked decrease in the development of diabetic eye, kidney and nerve complications in the people who received intensive therapy. During the first four years of the EDIC follow-up (1994 to 1998), the glycosylated hemoglobin of the two treatment groups became similar. On the basis of earlier epidemiologic studies, investigators expected to find that both groups of patients were experiencing diabetes-related eye and kidney complications at the same rate. Surprisingly, results indicated that people who had received intensive therapy during the DCCT continued to have a lower risk of eye and kidney disease than those who had been treated with conventional therapy. In the group who had received intensive therapy, the risks of progressive eye and kidney disease remained low, despite an increase in the glycosylated hemoglobin levels during the EDIC study. In contrast, in the group treated with conventional therapy, risk of progression of eye disease during the first four years of the EDIC study remained elevated at about the same level as during the first four years of the DCCT – despite that fact the glycosylated hemoglobin measurements actually declined during the EDIC study.

Implications: The results of the EDIC study demonstrate that the marked reduction in the risk of progressive eye and kidney diseases in those who received intensive therapy during the DCCT persisted for at least four years – despite increases in glycosylated hemoglobin. These findings suggest that early initiation of intensive therapy, continued for as long as possible, will have a beneficial effect on the long-term eye and kidney complications resulting from diabetes. Although this study included only patients with type 1 diabetes, the results are also likely to be applicable to those with type 2 diabetes.

The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The New England Journal of Medicine, 342(6):381-9. 2000.

Prevalence of Autoantibodies Against Contractile Proteins in Coronary Artery Disease

Background: Autoantibodies are antibodies produced by the body's own immune system against specific parts of its own body. Autoantibodies against actin and myosin (proteins that may be exposed in damaged heart muscle) and autoantibodies against troponin (a complex globular protein involved with heart muscle contraction), are found to be elevated in patients who have had a recent acute coronary syndrome, which includes pain in the heart region due to constriction of coronary blood vessels.

Advance: This study, supported in part by the Mt. Sinai School of Medicine General Clinical Research Center, theorizes that after a sudden cardiac event, such as unstable angina or ischemia (constriction of the coronary blood vessels), heart cells are damaged and release both actin and myosin. Since actin and myosin are not normally in the blood stream, the body's immune system recognizes them as foreign or non-self and produce antibodies against them. These autoantibodies can easily be detected in laboratory tests as can antibodies against troponin. The results show that, in 33 patients followed from the time of the acute cardiac event to three months following the event, all three autoantibodies were elevated. In patients with higher levels of elevation of these three autoantibodies, there was a higher risk of a later myocardial infarct or heart attack. Alternatively, the data also suggest that elevated levels of these autoantibodies may represent markers of earlier or ongoing heart tissue damage due to insufficient blood supply or inflammation, such as atherosclerosis.

Implications: The development of tests to identify autoantibodies against heart muscle proteins marks an important milestone in monitoring impending future cardiovascular events. Such information about a patient's likelihood of another attack can be used to set up preventive measures.

Dangas G, Konstadoulakis MM, Epstein SE, et al: Prevalence of autoantibodies against contractile proteins in coronary artery disease and their clinical implications. The American Journal of Cardiology, 85(7):870-2. 2000.

Neural Circuitry of Emotion Regulation

Background: After many years of relative neglect, neuroscientists are again embracing emotion as a legitimate research topic. Studies are now investigating how the brain processes emotional information and how emotions play a critical role in cognitive processes such as decision making.

Advance: A team of scientists has developed a theory called the "somatic marker hypothesis" that helps explain how the brain makes decisions and how emotions influence that process. According to this model defects in emotion and feeling play an important role in impaired decision making. "Somatic" here refers to the changes in body state – or their representation in the brain – which the brain monitors. The influence of emotions can occur at multiple levels, both consciously and non-consciously. Several areas of the nervous system including the ventromedial prefrontal cortex, the somatosensory/insular cortex, the anterior cingulate cortex, the amygdala and the peripheral nervous system work in concert in this large-scale system. The theory is based on a series of studies, conducted over several years, that combined use of neuropsychological tests, with physiological measures, and with neuroimaging for precise localization of brain lesions, in both normal and brain damaged people. Among the findings arising from this work, are demonstrations that damage to specific parts of this brain system involved with emotions may cause difficulties with tasks usually thought of as cognitive, such as probabilistic card games, or problems with acquisition of knowledge about accepted standards of moral behavior, despite apparently normal intellect. For example, previously well adapted individuals with damage to certain areas of the brain become unable to observe social conventions or to decide advantageously on matters pertaining to their own lives, despite well preserved learning, memory, language, attention, and other intellectual functions.

Implications: Theses findings have direct implications for understanding several psychopathologies that include depression, schizophrenia, pathological gambling, attention deficit and hyperactivity disorder (ADHD), and for many serious consequences of brain injury in children and adults. This line of work is also providing insights into the biological mechanisms of normal cognition and emotion.

Anderson SW, Bechara A, Damasio H, Tranel D, Damasio AR: Impairment of social and moral behavior related to early damage in human prefrontal ortex. Nature Neuroscience, 2(11):1032-7. 1999.

Bechara A, Damasio H Damasio AR: Emotion, decision making and the orbitofrontal cortex. Cerebral Cortex, 10(3):295-307. 2000.

Adolphs R, Damasio H, Tranel D, Cooper G, Damasio AR: A role for somatosensory cortices in the visual recognition of emotion as revealed by three-dimensional lesion mapping. The Journal of Neuroscience, 20(7):2683-90. 2000.

Understanding the Complex Genetics of Multiple Sclerosis

Background: The susceptibility to multiple sclerosis (MS) may be inherited, but the genetics of this disorder are probably complex, involving several genes working in concert with environmental factors. The complexity may reflect multiple susceptibility genes in a given individual (polygenic inheritance), different genes in different patients (locus heterogeneity), and possibly more than one underlying cause (etiological heterogeneity). Fully characterizing the genetic factors operating in this disease is an important goal.

A whole genome screen, originally published in 1996, identified 19 chromosomal regions that may harbor MS susceptibility genes. Two of these regions – one on chromosome 6 and the other on 19 – have been confirmed in follow-up studies as containing an MS gene or genes. Scientists are examining each of these regions in detail in order to determine the exact location of the genes of interest. A third region, in chromosome 3 includes the chemokine receptor 5 gene which scientists recently found is associated with a significant delay in age of disease onset.

Advance: The immune system plays a critical role in the progression of MS, and the chromosome 6 region implicated in MS includes the HLA region which codes for proteins found on the surfaces of cells that are critical in the immune response. Scientists carried out a detailed "saturation screen" of this region in a large group of families who had multiple members affected by MS, suggesting a genetic influence. The families were divided according to the presence or absence of the DR2 allele, one of the variant forms of HLA that people inherit. A consistent association between HLA-DR2 and MS had been previously observed in multiple studies of Caucasian MS populations. This new study found a strong link of the HLA region to MS susceptibility in the DR2 positive families, but not in the DR2 negative subset of families. These data demonstrate that genetic linkage to the HLA region can be explained by the HLA-DR2 allelic association, and suggest that sporadic and familial MS share a common genetic susceptibility. Furthermore, the results indicate the presence of locus heterogeneity in MS, with one form that is DR2-associated, and another that is DR2-negative and influenced by other genes or factors.

Implications: These data indicate that there is an underlying genetic heterogeneity in MS, supporting inferences from studies of pathology, neuroimaging and immunology suggesting that MS may result from more than one underlying biologic process. Studies are now underway to determine whether DR2 positive and DR2 negative MS populations differ in terms of their clinical manifestations or response to treatment. More generally, complex genetic influences probably contribute to most common neurological disorders, and these results are among the first indications that scientists are beginning to come to grips with understanding those influences.

Hauser SL, Oksenberg JR, Lincoln R, et al: Interaction between HLA -DR2 and abnormal brain MRI in optic neuritis and early multiple sclerosis. Neurology, 54(9):1859-61. (2000).

The Multiple Sclerosis Genetics Group: Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. Human Molecular Genetics, 7(8):1229-34. 1998.

Neurocognitive Phenotype in Turner Syndrome Mapped to a Critical Region of the X Chromosome

Background: There is considerable evidence that many cognitive and behavioral characteristics and abnormalities are genetically determined, but the pathway between genes and behavior is exceedingly complex. The study of human behavioral phenotypes, or characteristic patterns of cognitive, linguistic, social, or motor behavior that are consistently associated with a genetic or chromosomal disorder, may help us identify some of the specific genes involved. Turner syndrome (TS) is a chromosomal disorder in females who have only one X chromosome instead of two. In addition to some physical characteristics, many TS females have a typical cognitive profile, characterized by normal verbal but relatively poor visuospatial abilities. Although there are thousands of genes on the X chromosome, those likely to be responsible for the physical and cognitive symptoms of TS are among a much smaller number for which two copies are actually necessary. In males, homologous sites on the Y chromosome supply the needed second copy of these genes.

Advance: A team of scientists has identified a sample of Turner syndrome subjects whose symptoms resulted from partial deletions rather than a total absence of their second X chromosome. Subjects were given a comprehensive test battery to determine if they had the typical Turner syndrome neurocognitive profile. Only subjects whose deleted regions included a 10 megabase (Mb) region at the distal end of the short arm of the chromosome showed this phenotype, including five of seven subjects with very small (<2 Mb) deletions at the tip of the chromosome. This region, known as the pseudoautosomal region because of homologous sites on the Y chromosome, contains only seven known genes. Although the etiology of specific cognitive phenotypes probably involves complex interactions between genetic abnormalities and other influences, the authors concluded that missing a second copy one or more of these genes is the basis for susceptibility to the Turner syndrome neurocognitive profile. Narrowing the chromosomal location for a behavioral phenotype down to a region containing only seven genes represents a significant achievement. Future studies of the expression of these genes in humans or animal models will be needed to identify the specific genes and to understand the gene to behavior pathway.

Implications: Understanding the specific genes involved in Turner syndrome may lead not only to a better understanding of the underlying causes of this disorder, but also to important clues about the links between specific aspects of brain physiology and other behavioral problems.

Ross JL, Roeltgen D, Kushner H, Wei F, Zinn AR: The turner syndrome-associated neurocognitive phenotype maps to distal Xp. American Journal of Human Genetics, 67(3):672-81. 2000.

Parkinson's Disease is Not Just a Brain Disease

Background: Parkinson's disease progressively robs people of their ability to control movement. The cardinal symptoms are tremor, rigidity, slowness, and difficulty initiating movement. These problems reflect the loss of nerve cells that produce the neurotransmitter dopamine in a particular part of the brain.

People coping with Parkinson's must also confront a wide range of symptoms beyond disruption of movement, and the mechanisms of these non-motor problems are much less understood. Non-motor problems may include, for example, dementia, sleep disturbances, depression, swallowing problems, and sexual dysfunction. Some non-motor symptoms involve malfunction of the sympathetic nervous system. The sympathetic nervous system is part of the body's "fight-or-flight" stress response network that controls the cardiovascular system and other crucial bodily processes. Norepinephrine, a chemical closely related to dopamine, is a crucial neurotransmitter in the sympathetic nervous system. While disturbances of the sympathetic system are commonly observed in Parkinson's disease, like other non-motor symptoms, their cause is obscure.

Advance: Researchers have now discovered that most patients with Parkinson's disease loose sympathetic nerve terminals, which use the neurotransmitter norepinephrine, in the heart. This was revealed when patients with Parkinson's disease who had a fall in blood pressure upon standing (orthostatic hypotension) underwent positron emission tomography (PET) scanning of the chest after injection of [18F] fluorodopamine, a drug developed in the NIH intramural research program to reveal sympathetic nerves to the heart. Virtually all Parkinson's patients, even those without orthostatic hypotension, showed some loss of sympathetic nerve terminals in the heart. In contrast, no normal volunteers or patients with multiple system atrophy (another neurodegenerative disease) showed loss of sympathetic nerve terminals. Researchers are now investigating whether loss of sympathetic nerve terminals also occurs in other parts of the body and may contribute to other non-motor symptoms of Parkinson’s disease.

Implications: These results indicate that Parkinson's disease features not only loss of dopamine cells in the nigrostriatal system of the brain, but also loss of norepinephrine cells in the sympathetic nervous system to the heart. Dopamine and norepinephrine are both members of a chemical class called catecholamines, and the body synthesizes these two neurotransmitters through the same enzyme pathway. Parkinson's disease therefore may reflect an abnormality of catecholamines in the brain and elsewhere in the body.

Goldstein DS, Holmes C, Li ST, Bruce S, Metman LV, Cannon RO III: Cardiac sympathetic denervation in parkinson disease. Annals of Internal Medicine, 133(5):338-47. 2000.

Understanding and Early Detection of Huntington's Disease

Background: The hallmark of Huntington's disease is "chorea," uncontrolled, often complex, involuntary movements. Just as important to patients, voluntary movement control is also impaired, and often mental processes as well. For more than a century scientists have known that the steady progression of this disease reflects the dying of nerve cells in a region of the brain called the basal ganglia. Although the discovery a decade ago of the gene defect that causes this inherited disease is leading to a better understanding of why nerve cells die, the normal role of the basal ganglia in movement control is poorly understood, as is the mechanism of disturbed control in Huntington's.

Advance: Scientists have now determined that disturbances in "error control" circuitry underlie the voluntary movement problems in Huntington's disease. In people with normally functioning basal ganglia the brain can continuously monitor voluntary movements and use feedback to keep them on target. People with Huntington's disease lose the ability to make these compensations. Most importantly, subtle tests of movement control can reveal these deficits up to seven years before overt symptoms of the disease appear.

Implications: In many neurodegenerative diseases, including Huntington's, Alzheimer's and Parkinson's, nerve cells in the brain begin to sicken and die long before symptoms of the disease are detectable, and similar underlying processes contribute to nerve cell death in all these disorders. Given the availability of genetic tests to show who will get the disease, and now means to catch the process early, Huntington's may be one of the most favorable diseases for evaluating drugs to slow neurodegeneration. More generally, behavioral approaches may help in the early detection of many neurodegenerative diseases. Beyond the issue of early detection, a better understanding of how the basal ganglia contribute to movement control is critical for progress in several disorders that compromise this structure.

Smith MA, Brandt J, Shadmehr R: Motor disorder in Huntington's Disease begins as a dysfunction in error feedback control. Nature, 403(6769):544-9. 2000.

Different Populations Have Different Rates of Total Hip and Total Knee Replacements

Background: Osteoporosis is a common, chronic, and degenerative disease. It is one of the leading causes of long-term disability in the United States. Although it is not currently preventable, the pain and disability that result from severe disease can be reduced dramatically through the surgical implantation of artificial joints. Outcomes research has shown that total joint replacement can restore individuals with severe osteoarthritis of the hip and knee to pain-free functional independence. Studies of Medicare beneficiaries have documented racial and geographic differences in total hip and total knee arthroplasty.

Advance: A recent study focused on hip replacement for osteoarthritis in Hispanic (Mexican-American) populations found that Hispanics were underrepresented compared to both Hispanics with knee replacement for the same disease or other hospitalized persons. This pattern persisted even after adjusting for age, sex, type of medical insurance, and median household income. These data suggest that recipients of hip replacements are less likely to be Hispanic than are other persons with similar levels of access to care. Reasons for the disparity are unclear.

Implications: There appear to be substantial differences between ethnic groups in the utilization of joint replacements, with rates of utilization being higher in whites than among minorities. Hispanics, a minority U.S. population that is rapidly increasing, appear to be particularly underrepresented among recipients of hip replacements.

Escalante A, Espinosa-Morales R, Del Rincon I, Arroyo RA, Older SA: Recipients of hip replacement for arthritis are less likely to be hispanic, independent of access to health care and socioeconomic status. Arthritis and Rheumatism, 43(2): 390-9. 2000.

Teen-Aged Girls With Juvenile Rheumatoid Arthritis Have Risk Factor for Osteoporosis

Background: Fifteen to twenty percent of children with juvenile rheumatoid arthritis (JRA) fail to develop strong bones as demonstrated by a lower bone mass than their peers.

Advance: Researchers examined a homogeneous group of white teen-aged girls with JRA and a control group without JRA. None of the girls with JRA had been treated with glucocorticoids, so there was no adverse drug effect on bone mass. The researchers found that 70 percent of the girls with JRA had normal bone mass for their age, but a subset had low bone mass. They were shorter and weighed less, reflecting both lower lean body mass and percent body fat. However, their diets had more calories, protein, calcium, phosphorus, and magnesium than did those of the girls with JRA and normal bone mass, so the lower bone mass did not reflect a deficient diet. Lower lean body mass was found to be the best predictor of low bone mass.

Implications: Girls with JRA and a low lean body mass are at risk for low bone mass, and low bone mass is a risk factor for osteoporosis. Those being treated with glucocorticoids, drugs that tend to cause low bone mass on their own, are at even greater risk. As new agents become available for treating or preventing osteoporosis, some girls with JRA may be candidates for treatment.

Henderson CJ, Specker BL, Sierra RI, Campaigne BN, Lovell DJ: Total-body bone mineral content in non-corticosteroid-treated postpubertal females with juvenile rheumatoid arthritis: frequency of osteopenia and contributing factors. Arthritis and Rheumatism, 43(3):531-40. 2000.

The Influence of Stereotypes on Cardiovascular Health and Cognitive Functioning

Background: Researchers have focused on how negative stereotypes of aging influence younger people's perceptions of and behavior toward older people; behaviors which include patronizing speech, hiring discrimination, and bias when offering medical treatment options. Demographic trends associated with intergenerational competition for valuable resources suggest that the consequences of these widespread negative images may continue if nothing is done to counter this view. Researchers have now shown that unconscious exposure to both negative and positive age-related stereotypes impact memory and physical performance in older adults.

Advance: Findings from this study indicate that exposure to negative stereotypes of aging (older adults' beliefs about elderly people) contribute to adverse health outcomes; even when the individual is not consciously aware of such exposure. Experimental results with older adults demonstrate that unconscious negative stereotypes elicit cardiovascular stress (increased systolic and diastolic blood pressure and heart rate; precursors of heart disease) whereas positive stereotypes protect older adults from such stress. Timed tests of mathematical computation were administered after stereotype exposure. Those older adults exposed to positive stereotypes not only showed higher confidence in their ability to perform computations than those exposed to negative stereotypes but outperformed them as well. This study demonstrates the powerful influence of stereotypes on older adults' performance on tasks known to have age-related changes.

Implications: This research establishes a link between the cultural environment and health risk factors and cognitive performance. It offers a foundation for developing strategies to improve cognitive and physiological outcomes in older adults. Moreover, it points to physiological mechanisms that may explain negative health and performance disparities found among other populations who are frequent targets of negative stereotypes. Finally, these findings suggest that positive age-related stereotypes could be useful in interventions aimed at reducing cardiovascular response to stress, improving cognitive performance and performance on instrumental activities of daily living for older adults.

Hausdorff JM, Levy BR, Wei JY: Reducing cardiovascular stress with positive self-stereotypes of aging. Journal of Gerontology, Psychological Sciences, 55B:P205-13. 2000.

Mortality Continues to Decline in Industrialized Countries

Background: Throughout the past 150 years and particularly during the twentieth century, mortality rates have shown steady and significant declines in the G7 countries of Canada, France, Italy, Germany, Japan, the United Kingdom, and the U.S. During the first half of the twentieth century much of that decline has been attributed to decreased death rates among children due to a decline in the occurrence and spread of infectious diseases and improvements in nutrition. During the second half of the century, mortality decline has occurred most significantly in older populations due to greater health in later years, decreases in degenerative diseases (heart attack and stroke), and more recently, decreased mortality due to cancer. These "mortality shifts" have combined to sustain a long-term decline in mortality. Understanding the implications of such decline for future projections, however, remains uncertain.

Advance: Examining mortality data of the G7 industrialized countries over the last five decades, researchers found that long-term patterns in mortality rates have continued to decline exponentially at a remarkably constant rate, without evidence of slowing. More significantly, the findings of this research suggest that official estimates of longevity in the G7 countries underestimate life expectancy anywhere from 1.3 (United Kingdom) to 8 years (Japan). As a result, official estimates also understate the "dependency" or "support" ratio (the ratio of people over 65 to working people); a term helpful in estimating the social and monetary costs of increased longevity (i.e., Social Security, medical care, health insurance). Employing newer demographic modeling techniques their findings suggest that by the year 2050 these ratios may be between 6 percent (UK) and 40 percent (Japan) higher than official projections. This, taken together with investments to improved public health, is expected to result in sustained rates of mortality decline.

Implications: Official estimates of life expectancy from the G7 countries have consistently been too conservative, especially for Japan. With lower death rates, population aging in the G7 countries could continue to increase faster than expected, resulting in much higher numbers of persons over the age of 65 than officially projected. The findings of this research have significant implications for public policy regarding old-age support and underscore the need for capturing a more realistic picture of demographic shifts. Better estimates of life expectancy and future health conditions will facilitate more targeted planning for meeting medical needs of the elderly, as well as demands for long term care, retirement support, and other services.

Tuljapurkar S, Li N, Boe C: A universal pattern of mortality decline in the G7 countries. Nature, 405(6788):789-92. 2000.

Horiuchi S: Demography: greater lifetime expectations. Nature, 405(6788):744-45. 2000.

Environment and Not Heredity is the Overwhelming Contributor to Cancer Among Twins

Background: The contribution of hereditary factors to the causation of sporadic cancer is unclear. Studies of twins make it possible to estimate the overall contribution of inherited genes and environments to variation in the risk of developing malignant diseases.

Advance: Combining data from population-based twin registries and well-established cancer registries in three Nordic countries (Sweden, Denmark, and Finland), researchers assessed the risk of cancer at 28 anatomical sites for the twins of persons with cancer. Across all sites the rates of twin concordance for cancer were generally below 0.10. This result indicates that, for nearly all sites, the twin of a person with cancer has only a moderate absolute risk of having cancer at the same site. Biometric modeling procedures were used to estimate the relative contribution of genetic, shared and nonshared environmental factors to the risk of developing cancer. The shared environmental component includes influences and exposures that are common to both twins whereas the nonshared influences refer to any environmental cause of cancer not shared between twins. The contribution of these three sources of influence varied dependent upon the type of cancer, however, for all cancers nonshared environmental effects explained the greatest amount of variation in liability to disease, ranging from 58 to 82 percent. Thus, researchers conclude that environmental influences play a principal role in causing sporadic cancer and that inherited genetic factors make a minor contribution to susceptibility to most types of neoplasms. The relatively large effect of heritability for cancer at specific sites (prostate, colorectal, and breast cancers), coupled with prevalence information of inherited familial cancer syndromes suggests major gaps in our knowledge of the genetics of cancer. Most likely there are predisposing genes that are not yet identified each of which confer only a moderate risk for developing cancer. These results highlight the importance of identifying specific environmental factors, and the gene-environmental interactions in the pathogenesis of cancer.

Implications: Increasing knowledge regarding environmental and genetic influences causing cancer has major implications for the general population. The results of future cancer research in twin studies could be valuable in providing clinical guidance not only to the twins of persons with cancer but also to other first-degree relatives.

Lichtenstein P, Holm NV, Verkasalo PK, et al: Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from sweden, denmark, and finland. The New England Journal of Medicine, 343(2):78-85. 2000.

Early-life Childhood and Environment are Linked to Risk of Alzheimer's Disease

Background: The early-life environment and its effect on growth and maturation in children and adolescents are increasingly implicated as risk factors for many adult chronic diseases. Alzheimer's disease (AD) may also have an early-life link, involving such factors as nutrition and low levels of education, which may be associated with poverty, and other types of childhood exposures and deprivations.

Advance: One study looked at the association of early-life factors with AD including mother's age at patient's birth, birth order, number of siblings, and area of residence prior to age 18. Patient education level and versions of the APOE gene, some of which are risk factors for AD, were also included in the analyses. Results indicated that increased number of siblings was associated with an increased risk of AD and growing up in the suburbs was associated with a decreased risk. These associations were not modified by patients' educational level or APOE status.

A second study is part of a longitudinal epidemiological study of dementia and AD in a cohort of African Americans, 65 years of age and older. The purpose of the study was to evaluate the possible interactions between rural residence and low education on the risk of AD in this group of African Americans. The results indicate that for individuals who grew up in an urban setting, low education did not increase the risk of AD significantly. However, for those with rural residence to age 19, low education (less than or equal to 6 years of school) was a significant risk factor for AD.

Implications: As in other adult chronic diseases, the early-life childhood and adolescent environment is associated with the risk of developing AD. The results of these studies demonstrate associations that are consistent with socioeconomic or environmental variables perhaps altering brain growth and development, which in turn might affect the risk of developing AD.

Moceri VM, Kukull WA, Emanuel I, van Belle G, Larson EB: Early-life risk factors and the development of alzheimer's disease. Neurology, 54(2):415-20. 2000.

Hall KS, Gao S, Unverzagt FW, Hendrie HC: Low education and childhood rural residence - risk for alzheimer's disease in african americans. Neurology, 54(1):95-9. 2000.

Family Decision-Making to Withdraw Life-Sustaining Treatments from Hospitalized Patients

Background: Life expectancy in the United States has reached an all time high, but is accompanied by an increase in the number of people living with, and dying from, chronic debilitating diseases such as heart disease, cancer, stroke and chronic obstructive pulmonary disease. While the elderly with chronic illnesses comprise a group one might associate with end-of-life issues, there are other groups at all ages for whom these concerns are important. Coupled with this spectrum of individuals is the increased availability of technologies and treatments that can be used to prolong life and, in some cases, death. Defining when these technologies and treatments shift from life saving interventions to burdensome and futile procedure that negatively impact quality of life has been elusive. When these technologies and treatments become futile, the individuals' families and significant others may be involve in a difficult period of decision-making about how much aggressive treatment to try and when to stop. Conversely there is widespread fear that the only alternative to aggressive treatment is abandonment and suffering.

This study was a multi-method exploration of the factors contributing to stress among family members of patients in whom support was withdrawn. Data were collected from hospital records of people who had life support interventions withdrawn; from their family members at two times; and from care providers who were central to patient care and the decision to withdraw support.

Advance: Compared to other types of stressful situations reported in the literature, the level of family stress in the situation of deciding to withdraw life sustaining technology was higher. Three things intensified the stress level: absence of an advance directive, being an ethnic minority and commute distance from the hospital. The patients' values and preferences were the most important factor in making the decision to withdraw support. This study is one of the first to show that the existence of an advance directive, whether written or verbal, eases the burden for the family and reduces the stress associated with the decision to withdraw support.

Implications: With an advance directive to guide decisions, families were more able to focus on the patients' quality of life and less likely to endorse prolonging life at all costs.

Tolle SW, Tilden VP, Rosenfeld AG, Hickman SE: Family reports of barriers to optimal care of the dying. Nursing Research, 49(6):310-7. 2000..

Compare Preventive Interventions for Breast and Ovarian Cancer

Background: Even though heart disease is the leading cause of death for women, the disease that women fear most is breast cancer. Emphasis on early detection and treatment of breast cancer has been the focus of efforts by public and private sector groups as well as by the media. Identification of two genes that are associated with increased risk for breast cancer offered hope that at risk women would receive care necessary care to prevent, diagnose, treat and possibly cure breast cancer. As with many advances in medical technology, ethical dilemmas accompany the advances. One such dilemma is the set of ethical issues surrounding the conduct of randomized clinical trials of interventions to prevent breast cancer in women who are at genetically predisposed to developing breast cancer.

The randomized clinical trial is the gold standard used to evaluate the efficacy of a treatment or intervention. One possible barrier to conducting randomized clinical trials in women known to be genetically predisposed to breast or ovarian cancer may be their unwillingness to consent to randomization, since a woman's unique characteristics may make one arm of the clinical trial more preferable than another. The purpose of this study was to assess the feasibility of conducting randomized clinical trials for breast and ovarian cancer risk reduction in women with a family history of breast cancer who were participating in an educational offering about BRCA1/2 genetic testing.

Advance: Only a small percentage of women (17-19%) expressed willingness to participate in randomized trials to study risk factor reduction, whereas the majority were willing to participate in non-randomized trials. Women who expressed willingness to participate in a randomized trial were also more willing to undergo prophylactic mastectomy and were more likely to have children. Data suggested the type of prophylactic surgery (mastectomy versus hysterectomy) did not influence the willingness to participate in a randomized trial. The recommendations that physicians give to at-risk women are likely to vary depending on the types of providers. Oncologists were most likely and general practitioners were least likely to recommend randomized studies.

Implications: Investigators interested in conducting randomized clinical trials for breast and ovarian cancer risk reduction are likely to encounter difficulty recruiting eligible women. Non-randomized trials are more feasible and involve fewer ethical concerns. Therefore, this type of study is a more viable alternative to randomized trials for evaluating prevention interventions for breast and ovarian cancer when prophylactic surgery is one of the treatments being evaluated.

Tambor ES, Bernhardt BA, Geller G, et al: Should women at increased risk for breast and ovarian cancer be randomized to prophylactic surgery? an ethical and empirical assessment. Journal for Women's Health and Gender-Based Medicine, 9(3):223-33. 2000.

The Evolution of Bacterium-Human Interactions: The *H. pylori* Model

Background: Helicobactor pylori, a bacterium that is carried by more than half of all people world-wide, has attracted attention as a major cause of peptic ulcer disease and an early risk factor for gastric cancer. It is one of the most genetically diverse of bacterial species, with any given isolate easily distinguished from most others by DNA fingerprinting

Advance: In an NIH-supported study, researchers from Washington University Medical School in St Louis, and the Universidad Peruana Cayetano Heredia in Peru studied more than 500 strains of H. Pylori form 11 countries to gain insights into the evolution of this gastric pathogen and to identify new factors that affect colonization of disease in peoples of particular ethnicity. Their findings suggest that the microbe probably jumped to humans from animals only 10,000 years ago, about the time of animal domestication, and that travelers around the globe spread it. They identified three major strains of H. Pylori: Type I predominating in southern Europe, the Americas and Africa; Type II in the Far East; and Type III in India. They found that of 68 Peruvians and 27 Guatemalan participants, nearly all had Type I, a few had Type III but none had Type II, even though many had Native American ancestors and likely descended from Far Eastern peoples.

Implications: These findings suggest that European conquistadors first brought H. pylori to the Americas in the 1500s. Further analyses of strains from relatively understudied geographic regions and human ethnic groups may uncover new genes that affect the establishment of human infection, and increase our understanding of bacterium-human host interactions in colonization and disease.

Jeong JY, Mukhopadhyay AK, Dailidiene D, et at: Sequential inactivation of *rdx*A (HP0954) and *frx*A (HP0642) nitroreductase genes causes moderate and high-level metronidazole resistance in *Helicobacter pylori*. Journal of Bacteriology, 182(18):5082-90. 2000.

Kersulyte D, Mukhopadhyay AK, Velapatino B, et al: Differences in genotypes of *Helicobacter pylori* from different human populations. Journal of Bacteriology, 182(11):3210-8. 2000.

Mukhopadhyay AK, Kersulyte D, Jeong JY, et al: Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. Journal of Bacteriology, 182(11):3219-27. 2000.

Nitric Oxide Inhalation in Patients with Sickle Cell Anemia

Background: Sickle cell anemia is one of the most prevalent inherited diseases. Significant problems in a number of organs, including the lungs, can occur when there is sickling of the red blood cells, caused by a number of things including low levels of oxygen in the blood, dehydration, and infection. These sickled cells can alter flow in blood vessels, and in the most extreme circumstances this can lead to premature death. This severe sickling with organ damage produces sickle cell "crisis." Nitric oxide is a naturally occurring gas in the body that has effects on the walls of blood vessels to dilate them and to increase blood flow. Inhaled nitric oxide will bind to hemoglobin and can be released by the hemoglobin as it moves through the body. The nitric oxide can then increase blood flow to organs and other tissues. This therapeutic delivery of nitric oxide may be beneficial to patients with sickle cell anemia who have impaired blood flow in small blood vessels because of a direct effect on vasodilation (dilating of blood vessels).

Advance: Scientists have discovered that nitric oxide binds to hemoglobin in red blood cells in normal volunteers and in patients with sickle cell anemia during inhalation of nitric oxide gas, that it binds preferentially to the heme group of hemoglobin (where oxygen binds), and that, like oxygen, it is released as the hemoglobin moves through the body. Using special blood flow measurements, scientists have now shown that this released nitric oxide actually increases blood flow in the human arm. The scientists have also measured other chemical species of nitric oxide and have found that one of them, called nitrite, can be converted to nitric oxide in the body to increase blood flow as well. Ongoing studies are planned to see if patients with sickle cell anemia have an impaired ability to make nitric oxide and to determine if inhaled nitric oxide will increase their blood flow.

Implications: As mentioned earlier, patients with sickle cell disease suffer from blockages in their blood flow due to sickled red blood cells clogging the vessels. Therefore, the effect of inhaled nitric oxide on increasing blood flow hold therapeutic promise as a new treatment of sickle cell anemia. Other diseases such as coronary artery disease, diabetes, and hypertension are all characterized by a defective production of nitric oxide. These diseases could also potentially be treated with inhaled nitric oxide gas or with inhaled drugs designed to release nitric oxide.

Gladwin MT, Ognibene FP, Pannell LK, et al: Relative role of heme nitrosylation and ß-cysteine 93 nitrosation in the transport and metabolism of nitric oxide by hemoglobin the human circulation. Proceedings of the National Academy of Sciences, 97(18): 9943-8. 2000.

Gladwin MT, Shelhamer JH, Schechter AN, et al: Role of circulating nitrite and *S*-nitrosohemoglobin the regulation of regional blood flow in humans. Proceedings of the National Academy of Sciences, 97(21):11482-87. 2000.

Improving Functional Disability in Nursing Home Residents with Dementia

Background: Nursing home residents with dementia of the Alzheimer's type as a group have the most disabilities. Declines in their cognitive status contribute to these disabilities but other factors which are responsive to intervention may contribute. One factor is nursing staff reinforcement of residents' dependent behaviors. This hinders independent performance of daily activities, which can lead to excess disability beyond that expected from resident's level of cognitive impairment.

Investigators examined one-on-one interventions to improve the ability of cognitively impaired nursing home residents to perform bathing and dressing activities. The intervention involved a research therapist who first ascertained which bathing and dressing skills were retained by the resident with dementia. Then the therapist structured the physical and social environment to facilitate the resident being able to use those skills.

Advance: In response to the behavioral rehabilitation intervention, nursing home residents required less physical assistance with bathing and dressing activities, and the change was most dramatic for dressing. The decrease in physical assistance was accompanied by an increase in the number of verbal and nonverbal directions given to the resident and an increase in the number of requests the residents made for assistance with performing a task. These improvements in the ability of cognitively impaired nursing home residents were also accompanied by a reduction in the number of disruptive behaviors and behavioral indicators of distress such as rattling the bed rail. To achieve these improvements in performance of daily activities, the amount of time the care giver spent with the resident in performing the morning care activities of dressing and bathing doubled from 11 minutes to 20 minutes. These functional gains were attained within 5 days of the start of the interventions and were maintained over three weeks.

Implications: When care providers take the time to search for the skills that nursing home residents with dementia have retained, it is possible to reactivate those skills, promote more independent performance of morning care activities without creating disruptive behavior. However, these gains would require additional time on the part of the staff to search for and reward these behaviors.

Rogers JC, Holm MB, Burgio LD, et al: Improving morning care routines of nursing home residents with dementia. Journal of the American Geriatrics Society, 47(9):1049-57. 1999.

From Randomized Trial to Community-Focused Practice

Background: A concern of the NIH is finding ways to sustain intervention research in the community agencies where the studies were conducted. An NIH-funded, 5-year randomized clinical trial investigated telephone interventions by nurses to reduce the incidence of pre-term and low-birth-weight infants in a low income population. The intervention successfully reduced the incidence of low-birth weight infants in African American women in whom rates of preterm and low-birth-weight infants are double that of Caucasian women.

Following completion of the study the researchers worked with the agency to transfer this successful intervention to the community. The purpose of the paper was to describe the successful adaptation of the original research into four programs aimed at improving outcomes for pregnant women.

Advance: The first community program, the Good Health Program, was the original intervention at implemented the same clinic of low-income women that were studied in the original grant. A private foundation funded the implementation. This successful program was moving toward assuming responsibility for costs and operations when the clinic ownership changed, putting the future of the program in doubt. The second program was the Baby Watch program that was implemented through a health maintenance organization. When executives heard the results of the original study they wanted to use the intervention with their subscribers. This program focused on good nutrition and smoking cessation in the early trimester. The third program was Parent Line, a telephone intervention for parents of children between birth and five years of age. This program began in response to the women who were enrolled in the original study and was funded by a state initiative. The final program is Special Friends where African American women volunteer to have a one-to-one relationship with African American women who are pregnant. This program is funded by the medical school.

Implications: This is an example of how results from a research study can be successfully adapted and implemented into practice within the community. The transfer of research into practice needs to be considered when planning community-based intervention research.

Moore ML: From randomized trial to community-focused practice. Image: Journal of Nursing Scholarship, 31(4):349-54. 1999.

Activation of a Receptor Causes Abnormal Electrical Conduction in the Heart and a Lethal Heart Disease

Background: Dilated cardiomyopathy, a heart disease characterized by abnormal relaxation of the heart muscle and weakened pumping action, is a major cause of heart failure in the United States. While half of the cases are due to diseased heart arteries, the remainder, called idiopathic, have shown no discernable cause.

Advance: Investigators associated with the General Clinical Research Center at San Francisco General Hospital have shown a direct link between activation of a particular receptor on the surface of heart cells – known as the Gi-coupled receptor – and the development of most cases of the idiopathic form of dilated cardiomyopathy. Binding of naturally occurring or administered effector chemical compounds to various receptors on the surface of cells initiates a cascade of intracellular events that may be either beneficial or harmful, depending upon which member of the receptor family is activated. Gi receptor activation has been associated with heart failure but whether the activation caused the failure or was a secondary effect is unknown. Scientists using genetically manipulated mice have now shown that if the Gi receptor gene was continually activated in these mice, the animals developed cardiomyopathy and died within 16 weeks. At the same time their electrocardiograms became abnormal and showed irregularities similar to those in patients with cardiomyopathy. In contrast, if the Gi gene activation was suppressed, the disease progression was halted within 24 hours and the electrocardiogram became normal. Additional DNA structural studies have identified other genes that appear to be involved in this clinical syndrome. Knowledge of their role in the evolution of cardiomyopathy may improve our understanding and provide rational starting points for pharmacologic intervention.

Implications: The Gi pathway has now been shown to play a role in electrical conduction irregularities of the heart and cardiomyopathy and thus implicates an entire class of related receptors, providing insight into a wide variety of potential therapeutic targets for this syndrome. Furthermore, the identification of genes involved in the progression of cardiomyopathy should provide new diagnostic markers, such as secreted proteins, that could be measured in blood samples.

Redfern CH, Degtyarev MY, Kwa AT, et al: Conditional expression of a Gi-coupled receptor causes ventricular conduction delay and a lethal cardiomyopathy. Proceedings of the National Academy of Sciences, 97(9):4826-31. 2000.

SCIENCE CAPSULES

Gene Knockout has Implications for Alcoholism Treatment. Gene-knockout mice lacking the enzyme protein kinase C_e (PKC_e) are more sensitive to alcohol's effects and dramatically reduce their voluntary consumption of alcohol, new research reveals. PKC_e is among the enzymes that regulate activities in a receptor protein (the $GABA_?$ receptor) on nerve cells. A specific neurotransmitter binds to the $GABA_?$ receptor to deliver its chemical messages. Alcohol is known to amplify the $GABA_?$ receptor's activity, and evidence suggests that this amplification plays a role in whether or not an organism will seek additional alcohol. This new gene-knockout study suggests potential pharmaceutical applications for elimination or reduction of PKC_e activity, to increase $GABA_?$ receptor sensitivity, as a treatment for alcoholism and other conditions.

Hodge CW, Mehmert KK, Kelley SP,et al: Supersensitivity to allosteric $GABA_?$ receptor modulators and alcohol in mice lacking PKC_e. Nature Neuroscience, 2(11):997-1002. 1999.

Add Fruit Flies to the Search for the Genetics of Dopamine Response to Alcohol. Researchers have now shown that fruit flies are a useful model for tracking genes underlying neurobehavioral responses to alcohol and other substances of abuse known to be mediated, at least in part, by a major system of chemical message transmission in the brains of mammals,. The system involves the chemical messenger (neurotransmitter) dopamine and the nerve-cell proteins (receptors) that receive and respond to it, and play a major role in the biochemically "rewarding" properties of alcohol and other substances. To come to this finding, scientists first determined that the flies' behavioral responses to alcohol and other drugs were similar to those of mammals. Next, they used pharmacological agents to target and dampen the flies' dopamine neurotransmitter system. Researchers found that in fruit flies, too, the dopamine system is involved in regulating the behaviors. This finding suggests that a major pathway of addiction has the same biochemical basis in fruit flies as in mammals. Scientists can add this valuable new tool to their research on the genetics of dopamine's role in alcohol and other substances of abuse, a tool that is less complex and less expensive than that provided by mammal models.

Bainton RJ, Tsai LTY, Singh CM, Moore MS, Neckameyer WS, Heberlein U: Dopamine modulates acute responses to cocaine, nicotine, and ethanol in *Drosophila*. Current Biology, 10(4):187-94. 2000.

What Causes the Pleasurable Effects of Cocaine? While the brain chemical dopamine has been found to play an important role in mediating the pleasurable effects of virtually all drugs of abuse, evidence is emerging that other brain chemical systems or neurotransmitters, such as serotonin, may also play a role in causing pleasure. Serotonin is known for its role in regulating body temperature, sleep, sensory perceptions and impulse control. Serotonin acts in the brain by attaching to molecules

known as receptors. There are many receptors where serotonin can act. Using highly sophisticated bioengineering technology, scientists were able to produce a new strain of mice in which the gene that produces one type of serotonin receptor, called the 1B subtype, was missing or "knocked out." Researchers found that the knockout mice were more sensitive to the stimulant effects of cocaine and that the difference was related to increased dopamine in a particular brain area in these mice. This suggests that the serotonin 1B receptor subtype plays a critical role in regulating dopamine in the brain. Therefore, it appears that it is the interplay between these two neurotransmitter systems that contributes to the pleasurable effects of cocaine in these mice.

Shippenberg TS, Hen R, He M: Region-specific enhancement of basal extracellular and cocaine-evoked dopamine levels following constitutive deletion of the serotonin 1B receptor. Journal of Neurochemistry, 75(1):258-65. 2000.

Researchers Find Receptors for Molecules that Activate and Inhibit FSH. Researchers have proven the existence of cellular receptors for activin and inhibin, two molecules that switch on and off, respectively, the production of follicle stimulating hormone (FSH). Released by the pituitary, FSH significantly influences the functions of the ovary and testis, as well as affects the bone. Identifying these receptors will enable researchers to more fully understand the role of activin and inhibin in reproduction and in other biological systems, as well as better understand factors that may influence the development of certain gynecological disorders.

Lewis KA, Gray PC, Blount AL, et al: Betaglycan binds inhibin and can mediate functional antagonism of activin signaling. Nature, 404(6776):411-14. 2000.

Chong H, Pangas SA, Bernard DJ, et al: Structure and expression of a membrane component of this inhibin receptor system. Endocrinology, 141(7):2600-07. 2000.

Investigators Uncover How Substance Prepares Uterine Wall for Implantation. Researchers have found that Leukemia Inhibitory Factor (LIF) plays two important roles in early pregnancy – first in preparing the uterus to receive the blastocyst, and second in securing its successful attachment to uterine wall. This finding not only provides useful insights as to what uterine conditions are needed to establish a successful pregnancy, but may enable researchers to develop a diagnostic strategy to help assess a woman's fertility, or her ability to carry a fetus to term.

Song H, Lim H, Das SK, Paria BC, Dey SK: Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF deficient mice. Molecular Endocrinology, 14(8):1147-61. 2000.

Researchers Identify Gene for Sex Organ Development. Researchers have discovered a gene in mice that appears to control development of the sex organs. Genetically male mice lacking a functional *Lhx9* gene, a member of the gene family that regulates organ formation, appear to be female and female mice lacking the gene have underdeveloped female sex organs. Studies on these mice may provide important clues to human genetic diseases in which the sex organs fail to develop.

Birk OS, Casiano DE, Wassif CA, et al: The LIM homeobox gene Lhx9 is essential for mouse gonad formation. Nature, 403(6772):909-19. 2000.

Maternal Gene Found to be Essential for Early Embryonic Development in Mice. For the first time, researchers found that a gene in female mice, *Mater*, is required for early mouse embryos to continue developing beyond the point at which the mouse zygote splits into two cells. The mechanism by which this occurs is still not fully understood. Thus, researchers will study *Mater* to better understand the specific role it plays in early development. This in turn, may lead to new methods for treating infertility in women.

Zhi-Bin T, Gold L, Pfeifer KE, et al: *Mater*, a maternal effect gene required for early embryonic development in mice. Nature Genetics, 26(3):267-8. 2000..

Uncontrolled Harmful Protein Formation. Certain normal proteins in humans and other organisms, such as yeast, can assume shapes that are harmful and that reproduce themselves indefinitely. These wayward proteins, called prions, cause Mad Cow Disease and the human Creutzefeld-Jakob Disease. Investigators have discovered that a small portion of the prion protein can, if fused to an entirely unrelated protein, convert that protein to a prion as well. This information will be used by investigators to pin down the critical processes involved in prion formation, and how prion formation may be prevented or reversed.

Li L, Lindquist S: Creating a protein-based element of inheritance. Science, 287(5453):661-4. 2000.

Clues to the Development of Alzheimer's Disease. Molecular members of a biochemical pathway that leads to nerve cell death have been identified. This pathway, which may partially explain the development of Alzheimer's Disease in some individuals, involves regulation of an enzyme by a protein partner. When the normal protein partner is modified, the activity of the enzyme goes unchecked, leading to the formation of the plaques and tangles found in brain cells of Alzheimer's disease patients. The members of this pathway may be targets for drugs that could slow or halt the progression of neurodegenerative disorders, including Alzheimer's disease.

Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai LH: Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. Nature, 402(6762):615-21. 1999.

Vaults: Hollow Barrels or Treasure Chests. Vaults, the largest RNA-protein complex present in cells from sea urchins to mammals, are present at 10,000-100,000 copies per cell and have potentially exciting functions in multidrug resistance. Multidrug resistance occurs when some tumor cells become resistant to chemotherapy because they stimulate a pump to push the therapeutic drugs out of the cell. Lung and breast cancer cells activate a multidrug resistance-associated protein called Lung Resistance-related Protein, or LRP, which turns out to be the major vault protein (MVP), the dominant protein component of vaults. Two MVPs have been cloned and characterized, providing both information on what causes one MVP to activate, and leads as to the potential functions of each of these proteins.

Schroeijers AB, Siva AC, Scheffer GL, et al: The M_r 193,000 vault protein is up-regulated in multidrug-resistant cancer cell lines. Cancer Research, 60(4):1104-10. 2000.

Kong LB, Siva AC, Kickhoefer VA, Rome LH, Stewart PL: RNA location and modeling of a WD40 repeat domain within the vault. Ribonucleic Acid, 6(6):890-900. 2000.

Inhibitor Protein Works by Changing Shape. As the body grows and changes, and when wounds are healed, cells must be released from the glue that holds them in place and allowed to move – but not too freely, as they do in metastatic cancers. To maintain careful control of the ability of cells to migrate, the body has both a protein that frees cells (MMP) and also an inhibitor of MMP. A new three-dimensional structure shows that to stop the cell-releasing activity of MMP, the inhibitor protein must change to a different shape, which binds tightly to the MMP. Drugs that make this binding weaker or stronger could be important in the treatment of cancer and arthritis.

Wu B, Arumugam S, Gao G, et al: NMR structure of tissue inhibitor of metalloproteinases-1 implicates localized induced fit in recognition of matrix metalloproteinases. Journal of Molecular Biology, 295(2):257-68. 2000.

Structural Basis of DNA Synthesis. State-of-the-art biophysical techniques have been used to describe the molecular details of catalysis by DNA polymerase, an enzyme that synthesizes a new strand of DNA from an existing template. This basic cellular process takes place each time a cell divides and requires a high degree of fidelity. DNA polymerase is a highly complex enzyme that carries out at least two major functions: DNA replication and 'proof-reading and editing' an additional activity that discovers and removes misincorporated bases at a separate active site. The mechanical force generated during replication by DNA polymerase has been measured and, additionally, the precise 3-D

molecular structure of the editing complex has been defined. These studies give us fundamental new information about both the structural and dynamic features of a basic cellular process.

Wuite GJL, Smith SB, Young M, Keller D, Bustamante C: Single-molecule studies of the effect of template tension on T7 DNA polymerase activity. Nature, 404(6773):103-6. 2000.

Shamoo Y, Steitz TA: Building a replisome from interacting pieces: sliding clamp complexed to a peptide from DNA polymerase and a polymerase editing complex. Cell, 99(2):155-66. 1999.

Genetic Basis Found for Hypertension During Pregnancy. Hypertension during pregnancy is a major public health problem, the causes of which are still largely unknown. Scientists recently identified a genetic mutation as a cause of one type of pregnancy-exacerbated hypertension. The mutation affects how salt is absorbed in the kidney. This new understanding of salt handling in the kidney could lead to genetic tests to identify women at high risk for pregnancy-exacerbated hypertension and the common complication, pre-eclampsia, as well as new treatments for hypertension.

Geller DS, Farhi A, Pinkerton N, et al: Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. Science, 289(5476):119-23. 2000.

Possible Explanation Found for High Levels of Hypertension in Black Americans. Not only do blacks in the United States experience more hypertension and related kidney disease than members of other ethnic groups, but aggressive blood pressure control does not always lead to similar benefits for blacks as for whites. Recent study results show higher levels of a protein called transforming growth factor beta-1 (TGF-ß1) in hypertensive individuals than in individuals with normal blood pressure levels, and higher levels in blacks than in whites; the highest levels were found in blacks with hypertension. TGF-ß1 might explain the high levels of hypertension in black Americans, and might also lead to new treatment targets, as well as predictors, for hypertension and associated organ damage.

Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, August P: Transforming growth factor-ß1 hyperexpression in african-american hypertension: a novel mediator of hypertension and/or target organ damage. Proceedings of the National Academy of Sciences, 97(7):3479-84. 2000.

Understanding the Genetics of Pulmonary Fibrosis. Pulmonary fibrosis, characterized by the replacement of normal lung tissue by connective tissue, results in loss of lung function and death. About 100,000 people in the United States have pulmonary fibrosis – 50 percent die within 5 years of onset. Current therapies to inhibit the lung inflammation that proceeds fibrosis are effective in only a small percentage of cases and there are no treatments targeting the fibrotic process itself. Investigators were

able to identify the genes associated with inflammation and fibrosis in mice and the results of their genetic study were placed on the internet so other researchers would have access to the data. Understanding the genetic elements involved in the development of pulmonary fibrosis is a giant first step toward developing new treatment strategies, not just for this disease, but for those resulting in fibrosis of other organs as well.

Kaminski N, Allard JD, Pittet JF, et al: Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proceedings of the National Academy of Sciences, 97(4):1778-83. 2000.

Research on Rare Disease has Implications for Understanding Cancer and Normal Cellular Processes. Researchers recently identified a defect in the DNA repair process of Fanconi anemia (FA) cells. Because FA is associated with an increased risk for developing a variety of cancers, identifying its underlying mechanism is also helping researchers to understand the development of such cancers in patients who do not have FA. In addition to laying the groundwork for future cancer treatments, these results add to our understanding of the normal DNA repair mechanisms in healthy cells and ultimately may contribute to the development of cancer prevention efforts.

Kumaresan KR, Lambert MW: Fanconi anemia, complementation group A, cells are defective in ability to produce incisions at sites of psoralen interstrand cross-links. Carcinogenesis, 21(4):741-51. 2000.

Identification of Protein Responsible for Ebola's Devastating Effects Lays Groundwork for Vaccine Development. Ebola virus is a rare but deadly microbe that kills up to 90 percent of the people that it infects. Recently, scientists identified the viral gene thought to be responsible for the massive internal bleeding that leads to most of those deaths. Based on information about the gene and the protein that it produces, researchers have developed a vaccine that is being tested in animals prior to the initiation of vaccine trials in humans.

Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ: Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nature Medicine, 6(8):886-9. 2000.

DNA Repair – Integrated and Mulitstepped. The DNA that makes up our genes is constantly being damaged and repaired. DNA repair enzymes are very important and, if they do not operate correctly, can lead to such diseases as colon, breast, and prostate cancer. A new study suggests that the enzymes involved in these complex systems might not work independently. That is, when one enzymatic reaction is over, the product is presented to the next enzyme in a sequential, specific pattern. This has major implications for science and therapeutics. Holding on means that the partially repaired

DNA isn't loose in a cell, where it might actually be more harmful than it was before the enzyme worked on it. It appears that the individual chemical steps controlling genetic integrity are integrated like a dance where partner exchanges, steps, and timing are carefully coordinated.

Mol CD, Izumi T, Mitra S, Tainer JA: DNA-bound structures and mutants reveal abasic DNA binding by APE1 DNA repair and coordination. Nature, 403(6768):451-6. 2000.

Influence of Thyroid Hormones on Fetal Brain. Scientists have known for some time that thyroid hormone is essential for proper brain development, but the influence of thyroid hormone was thought to be after birth. By giving pregnant female rats large doses of a thyroid hormone known as thyroxine (T4) prior to fetal thyroid development, researchers were able to demonstrate expression of genes in the fetal brain tissue. This study is the first to identify thyroid hormone responsive genes in rodent fetal brain tissue.

Dowling ALS, Martz GU, Leonard JL, Zoeller RT: Acute changes in maternal thyroid hormone induce rapid and transient changes in gene expression in fetal rat brain. Journal of Neuroscience, 20(6):2255-65. 2000.

Prostate Cancer Predictor – Mutated Androgen Metabolism Genes. Metabolism genes for the male hormone, androgen, are thought to play a role in the development of prostate cancer. One such gene, SRD5A2, encodes for 5a-reductase type II, an enzyme that converts testosterone to a more metabolically active metabolite dihydrotestosterone (DHT). DHT has more androgen activity in the prostate than testosterone and when it is bound to the androgen receptor it activates genes involved in prostate development and growth. Therefore, SRD5A2 may be involved in prostate cancer development. These investigators studied the A49T and V89L polymorphisms or variants of SRD5A2 in prostate tumors removed from men. The presence of the A49T polymorphism was associated with several indicators of poor prognosis including advanced disease and greatly increased prostate specific antigen levels. These results suggest that the A49T mutation may affect the severity of prostate cancer and thus be a predictor of patient outcome.

Jaffe JM, Malkowicz SB, Walker AH, et al: Association of *SRD5A2* genotype and pathological characteristics of prostate tumors. Cancer Research, 60(6):1626-30. 2000.

Prostate Cancer – New Model for Studying Early Initiating Events. Human insulin-like growth factor 1 (IGF-1) is a naturally occurring cellular growth factor that has been implicated in a variety of epithelial cancers including skin cancer and prostate cancer. Using a transgenic mouse strain that expresses IGF-1 in prostate epithelial cells, investigators showed that activation of the IGF-1 receptor led to spontaneously induced prostate tumor formation. The mice developed increased prostatic growth

very early in life and tumor formation by 6 months of age. The development of tumors in the mice seemed to follow a stepwise pattern from early precancerous changes to obvious tumor formation. This model for prostate cancer will allow study of the progression of tumor formation and the role IGF-1 plays in prostate cancer development.

DiGiovanni J, Kiguchi K, Frijhoff A, et al: Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. Proceedings of the National Academy of Sciences, 97(7):3455-60. 2000.

Killing Cancer Cells. Cytotoxic endoribonucleases (RNases) are proteins that have potential uses in cancer chemotherapy because of their affinity for killing rapidly dividing cells with a certain preference for cancer cells. However, the mechanism by which cell death occurs is not fully understood. A new study, using an amphibian-derived RNase known as onconase, describes some of these intricate cellular events that lead to apoptosis or programmed cell death. Further work in this area may lead to a more detailed understanding of the cytotoxic action of onconase which could further its use as a therapeutic agent in fighting cancer.

Iordanov MS, Ryabinina OP, Wong J, et al: Molecular determinants of apoptosis induced by the cytotoxic ribonuclease onconase: evidence for cytotoxic mechanisms different from inhibition of protein synthesis. Cancer Research, 60(7): 1983-94. 2000.

Insight into Down's Syndrome – Possible Role of ITSN Protein. Scientists have demonstrated that the adaptor or scaffolding protein, intersectin (ITSN), is able to activate signaling pathways that lead to increased cell growth or mitogenesis. ITSN has also been shown to play an important role in regulating endocytosis, a process by which cells internalize molecules on the cell surface as well as in the extracellular milieu. These results provide evidence that endocytosis and mitogenesis are directly linked through ITSN. Since the ITSN gene is localized on human chromosome 21 in the so-called Down Syndrome region, these data provide additional support to the notion that improper expression of ITSN in Down Syndrome may play a potential role in a subset of the phenotypes associated with this genetic disorder. In addition, this work demonstrates that overexpression of ITSN is sufficient to induce transformation of cells to cancer, suggesting that this protein may play a role in malignant progression.

Adams A, Thorn JM, Yamabhai M, Kay BK, O'Bryan JP: Intersectin, an adaptor protein involved in clathrin-mediated endocytosis, activates mitogenic signaling pathways. The Journal of Biological Chemistry, 275(35):27414-20. 2000.

Increasing Production of White Blood Cells – New Line of Investigation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an important cytokine in the development of a specific population of white blood cells, the myeloid cells, and is used therapeutically in patients with low levels of these cells resulting from, for example, cancer chemotherapy. Researchers have discovered a novel mechanism by which production and secretion of GM-CSF is regulated. These workers found that a protein originally cloned in their lab, tristetraprolin or TTP, was responsible for causing instability of the mRNA encoding GM-CSF. Conversely, this provides a new drug target for these disorders, in that inhibitors of TTP action would help to increase the secretion of GM-CSF, and thus increase the formation of white blood cells.

Carballo E, Lai WS, Blackshear PJ: Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood, 95(6):1891-9. 2000.

Inhibiting the Inflammation Leading to Rheumatoid Arthritis and Crohn's Disease. Researchers have identified a small family of zinc finger proteins (areas within proteins that can mediate interactions with DNA, RNA, other proteins, and small molecules) that can inhibit the production of the inflammatory cytokine Tumor Necrosis Factor a (TNFa) from cells. Cytokines are proteins released by immune cells that signal other cells to perform specific functions. The particular cytokine studied here is involved in the harmful inflammatory aspects of many common human diseases, such as rheumatoid arthritis and Crohn's disease, an inflammatory disease of the intestinal tract. All of the members of this protein family can inhibit production of this cytokine from cells by destabilizing its mRNA. The discovery of this pathway may make possible the development of potentiating or mimicking drugs that could be used to treat inflammatory diseases.

Lai WS, Carballo E, Thorn JM, Kennington EA, Blackshear PJ: Interactions of CCCH zinc finger proteins with mRNA: binding of tristetraprolin-related zinc finger proteins to AU-rich elements and destabilization of mRNA. The Journal of Biological Chemistry, 275(23):17827-37. 2000.

Identification of a Gastrointestinal Tumor Suppressor Gene. Gastric cancer is a leading cause of morbidity and mortality worldwide. As in many types of tumors, the development and progression of gastric cancer is a multi-step process. Researchers recently demonstrated that one mechanism of tumor progression is the inactivation of the transforming growth factor (TGF) beta signaling pathway. TGF beta is a regulatory protein with diverse biological activities. The tumor suppressor gene, Smad4, is the central mediator of this pathway. Mice lacking the protein coded for by this gene have exhibited impaired embryonic membrane formation and tissue differentiation and died during embryonic development, preventing further studies during later developmental stages. Researchers next assessed the tumor suppressor function of Smad4 in mice lacking one normal allele. These mice developed

hyperplasia – an abnormal increase in the number of normal cells – within certain portions of the gastrointestinal (GI) tract. Hyperplasia of the fundal region, or the portion of the stomach to the left and above the entrance to the esophagus, rarely progressed into tumors. On the other hand, hyperplasia in the antrum, that portion of the GI tract between the body of the stomach and the opening to the intestine, eventually developed into tumors as the mice aged. However, loss of the remaining normal Smad4 allele was detected in these animals only in the later stages of tumor progression. These data indicate that Smad4 is a major tumor suppressor gene in the GI tract, especially in the stomach, and that loss of one normal allele is sufficient for tumor initiation. Data also demonstrated that over-production of TGF beta and other proteins was associated with increased cell number and eventual development of tumors, demonstrating the value of this model for screening factors that may promote or prevent the formation of tumors.

Xu X, Brodie SG, Yang X, et al: Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. Oncogene, 19(15):1868-74. 2000.

Simple System Yields Clues About Anemia. Zebrafish mutants are proving of great value in developmental and genetic studies. Investigators report identification of a mutant zebrafish gene that results in a type of anemia during embryonic development. The gene, named *ferroportin1*, produces a protein that abnormally transports iron out of cells rather than into cells. It is proposed that function of this newly discovered protein, which also is found in mammals, may be perturbed in disorders such as iron deficiency anemia or iron overload disorders, such as hemochromatosis.

Donovan A, Brownlie A, Zhou Y, et al: Positional cloning of zebrafish *ferroportin1* identifies a conserved vertebrate iron exporter. Nature, 403(6771):776-81. 2000.

AIDS Therapy May Promote Diabetes. Protease inhibitors – the main form of current treatment for AIDS – have been implicated in recent reports of fat redistribution, insulin resistance and increased blood lipid levels in HIV-infected patients receiving highly active antiretroviral therapy (HAART). Animals with an absence, or low levels, of a protein (glucose transporter 4) that facilitates transport and storage of glucose in fat and muscle cells are unable to store fat, respond poorly to insulin and are prone to develop type 2 diabetes. Investigators hypothesized that protease inhibitors act to disable this protein in HIV-infected patients. In order to validate this hypothesis, researchers studied the effect on fat cells of three common protease inhibitors, in concentrations found normally in patients receiving protease inhibitor therapy. They found that human fat cells absorbed less glucose after exposure to the drugs. In an attempt to decipher how these drugs disabled the protein, researchers analyzed the steps in the pathway that promote glucose storage. They found that the protease inhibitors acted directly to prevent the transport protein from functioning. These results suggest that HIV protease inhibitors block the

body's ability to store glucose, making treated individuals prone to developing diabetes. Based on these data, physicians may wish to rethink how they assess diabetes risk in patients receiving protease inhibitors, thereby preventing the development of a serious complication. This research also suggests a mechanism for developing new agents to treat HIV without diabetes risk. Researchers are now working to determine if blocking the body's ability to store glucose causes body fat redistribution in patients receiving protease inhibitors.

Murata H, Hruz PW, Mueckler M: The mechanism of insulin resistance caused by HIV protease inhibitor therapy. The Journal of Biological Chemistry, 275(27):20251-4. 2000.

Regulation of Protein Degradation. Ubiquitin is a protein that exists either in a free form or covalently joined to other proteins. Ubiquitination of a protein, or the covalent linkage of ubiquitin to the protein, targets that protein for selective degradation. This "ubiquitin-dependent" protein degradation pathway is responsible for the breakdown of most short-lived proteins and is vital in controlling the concentration of key regulatory proteins involved in signal transduction and the cell cycle. This pathway is also suspected to play a role in metabolism and the transport of small molecules across membranes. Ubiquitin selectively recognizes a protein via a feature of its structure. The rate of protein degradation may then be regulated by further modification of the protein structure, for example, through phosphorylation. In other cases, a modification such as phosphorylation regulates the activity of an enzyme called E3 that is directly involved in the ubiquitin-dependent pathway. Researchers recently described the regulation of a ubiquitin-dependent pathway through the binding of a dipeptide to a site on the E3 enzyme that is different from the functional binding site. Binding of this dipeptide increases degradation of a repressor of dipeptide transport by E3. This work establishes for the first time that the activity of the E3 enzyme can be directly linked to the presence of an environmental signal through an interaction with a small peptide. The data also suggest that small compounds may regulate other ubiquitin-dependent pathways.

Turner GC, Du F, Varshavsky A: Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway. Nature, 405(6786):579-83. 2000.

Prenatal Androgen Exposure May Affect Insulin-Glucose Balance in Adulthood. Women with polycystic ovarian syndrome (PCOS) exhibit disorders characterized by hormone imbalances that may affect insulin and glucose metabolism. Scientists used female rhesus monkeys to determine whether excessive exposure to male hormones – androgens – at different times during pregnancy would result in different types of insulin-glucose imbalance. The study showed that monkeys exposed to the hormone testosterone early in gestation had impaired function of insulin-producing cells, but those exposed to the hormone late in gestation had difficulty utilizing insulin. It is possible that prenatal androgen exposure in women can induce a PCOS-like condition without causing external genital virilization.

Eisner JR, Dumesic DA, Kemnitz JW, Abbott DH: Timing of prenatal androgen excess determines differential impairment in insulin secretion and action in adult female rhesus monkeys. The Journal of Clinical Endocrinology & Metabolism, 85(3):1206-10. 2000.

Zebrafish Gene Sheds Light on Embryonic Development of Blood Vessels. Arteries and veins are very distinct, both functionally and morphologically. These differences arise very early in development in order for the vessels to be able to cope with the output of the developing heart. By studying a mutation in zebrafish called *gridlock (grl)*, which selectively interferes with assembly of the aorta, the major artery of the body, researchers have found that arterial cell identity is established in the embryo prior to onset of blood flow and blood vessel formation. Other similar mutations may provide important information about how the different organs of the body are formed.

Zhong TP, Rosenberg M, Mohideen MAPK, Weinstein B, Fishman MC: *Gridlock*, an HLH gene required for assembly of the aorta in zebrafish. Science, 287(5459):1820-4. 2000.

Animal Models of Neurological Disorders. For neurological disease, the development of animal models of human disorders is one of the most important developments from research in molecular genetics. In the past year genetic research has let to new or better models for Canavan disease, spinal muscular atrophy, neurofibromatosis, and neural tube defects, to name a few. Intensive research is continuing using mouse models of Alzheimer's, amyotrophic lateral sclerosis, Batten disease, inherited ataxias, muscular dystrophies, and many other disorders. An important new trend is the advent of genetically engineered drosophila (fruitfly) models of human neurological disorders, including epilepsy, Parkinson's disease, neurofibromatosis, inherited ataxias, and several others, which offer substantial advantages over mammals for unraveling biochemical pathways. Mouse, fly, and other animal models are crucial for understanding disease mechanisms, identifying genes that might modify the process, and developing treatment strategies.

Matalon R, Rady PL, Platt KA, et al: Knock-out mouse for canavan disease: a model for gene transfer to the central nervous system. Journal of Genetic Medicine, 2(3):165-75. 2000.

Carter M, Ulrich S, Oofuji Y, Williams DA, Ross ME. *Crooked tail* (Cd) models human folate-responsive neural tube defects. Human Molecular Genetics, 8(12):2199-2204. 1999.

Guo HF, Tong J, Hannan F, Luo L, Zhong Y: A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. Nature, 403(6772):895-8. 2000.

Monani UR, Sendtner M, Coovert DD, et al: The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn –/– mice and results in a mouse with spinal muscular atrophy. Human Molecular Genetics. 9(3):333-9. 2000.

Warrick JM, Paulson HL, Gray-Board GL, et al: Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. Cell, 93(6):939-49. 1998.

Kuebler D, Tanouye MA: Modifications of seizure susceptibility in *Drosophila*. Journal of Neurophysiology, 83(2):998-1009. 2000.

Jackson GR, Salecker I, Dong X, et al: Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. Neuron, 21(3):633-42. 1998.

Mapping of a Gene for Severe Pediatric Gastroesophageal Reflux. Gastroesophageal reflux (GER) affects persons of all age groups and is characterized by the retrograde movement of stomach contents into the esophagus. Children with severe GER have an increased incidence of acute and chronic infections in the upper respiratory tract, which predispose to voice disorders. Although not previously regarded as a hereditary disease, a few reports have suggested that a genetic component may contribute to the incidence of GER. NIDCD researchers conducted a genome-wide scan of affected families in which severe pediatric GER followed an autosomal dominant hereditary pattern with high penetrance. A gene was mapped to chromosome 13q14. Ascertaining the genetic basis for GER is important in designing more effective, rational therapies, and understanding the physiological processes that underlie severe pediatric GER. Efforts to narrow the locus and identify the gene itself are underway.

Hu F,Preston RA, Post JC, et al: Mapping of a gene for severe pediatric gastroesophageal reflux to chromosome 13q14. Journal of the American Medical Association, 284(3):325-34. July 2000.

Molecular Motor Activity is Regulated by its Load. Much of the movement of organelles and substances within cells is based on structures known as microtubules. The movement is carried out by kinesins, molecules composed of two similar portions, each of which consists of a head region and a tail region. The head fuels cellular motion, and the tail binds to organelles or other substances being transported along the microtubule. Investigators have discovered that kinesin activity is sharply curtailed in the absence of a bound cargo. If the activity were to proceed in an uncontrolled fashion, considerable energy would be wasted. Insight into how cells regulate internal transportation for organelles and nutrients should enable design of agents that will block cell division in certain types of cancers and other diseases in which cells hyperproliferate.

Coy DL, Hancock WO, Wagenbach M, Howard J: Kinesin's tail domain is an inhibitory regulator of the motor domain. Nature Cell Biology, l(5):288-92. 1999.

Tissue Engineering Shows Promise for Articular Cartilage Repair. Articular cartilage injuries are frequent, surgically challenging, and despite the best treatment, sometimes progress to end-stage osteoarthritis primarily because of the inability of articular cartilage to heal itself. Tissue engineering shows great promise in the treatment of articular cartilage injuries. To better understand the molecular

and cellular events that occur in cartilage repair, investigators used tissue engineering in an in vitro rabbit model. Results suggest that BMP-2, a growth factor, plays an important role as a regulator of early events in cartilage repair. Future studies will be needed to determine at which point in the cascade of events in cartilage formation BMP acts, and which factors regulate it or are regulated by it.

Sanyal A, Sarkar G, Saris DBF, et al: Initial evidence for the involvement of bone morphogenetic protein-2 early during periosteal chondrogenesis. Journal of Orthopaedic Research, 17(6):926-34. 1999.

Establishment of Water Barrier of Skin Requires Coordination of Events. The major function of the outer layer of skin is as a barrier to the loss of water from the skin and to the intake of environmental agents. It is a protective device but does not limit the ability of topically applied medications to get into skin and exert a beneficial effect. The establishment of the water barrier requires a number of events to take place in a coordinated fashion. In a recent study, investigators demonstrated that selective blockage of one or more of the events results in disruption of the entire process. They then showed that the water barrier can be reestablished by the external supply of one or more of the missing elements. Understanding water barrier function at a molecular and cellular level allows interventions to reestablish this vital function when it is damaged and to selectively perturb it for the penetration of therapeutic agents.

Rassner U, Feingold KR, Crumrine DA, Elias PM: Coordinate assembly of lipids and enzyme proteins into epidermal lamellar bodies. Tissue and Cell, 31(5):489-98. 1999.

Novel Melanocyte Protein is Discovered. Pigmentation abnormalities represent a diverse group of diseases including hereditary and acquired. Some are peculiar to certain workplace exposures. Proteins specific to pigment-producing cells, melanocytes, have proven useful in designing experimental treatments for human melanoma. In recent research, investigators discovered a melanocyte-specific, not previously reported, protein that resides in the cell membrane. Its role is not yet known, but scientists are hopeful that it may prove useful in the study and treatment of melanoma. Understanding pigment disorders provides the basis for development of prevention strategies and new therapeutic approaches.

Samaraweera P , Donatien PD, Qazi S, et al: Identification and characterization of a melanocyte-specific novel 65-kDa peripheral membrane protein. European Journal of Biochemistry, 226(3):924-34. 1999.

Monoclonal Antibody Identifies Hair Follicle Stem Cells. Tissue-specific stem cells are those stem cells found in a specific tissue that retain the potential for long life and differentiation into the specialized cells of that tissue. They are important in understanding the biology of the specific tissue. Many attempts have been made to find markers to more readily identify stem cells in the hair follicle. In

a recent study, investigators using a monoclonal antibody that identifies keratin 15 (keratins are the major structural protein of hair) found that it also identifies hair follicle stem cells. This discovery makes it easier to identify stem cells and suggests that keratin 15 is preferentially produced by stem cells. Identification of skin stem cells is an important step in the design of many genetic and other therapies for skin disease, as well as in the use of skin as a factory for proteins to treat systemic diseases.

Lyle S, Christofidou-Solomidou M, Liu Y, et al: Human hair follicle bulge cells are biochemically distinct and possess an epithelial stem cell phenotype. Journal of Investigative Dermatology Symposium Proceedings, 4(3):296-301. 1999.

Role of Measles Virus in Paget's Disease of Bone Begins to Emerge. Bone breakdown, or resorption, is a normal part of bone remodeling, in which old or damaged bone is replaced with new bone. Cells that resorb bone are called osteoclasts; cells that form new bone are called osteoblasts. In Paget's disease of bone, both resorption and formation are excessive at specific sites in the skeleton (called pagetic lesions), leading to replacement of normal bone with bone that is of poor quality. Recent research findings argue strongly that viral infection (using genes from the measles virus) can be an important causative factor in Paget's disease. Although antiresorptive drugs can reduce the pathological manifestations of the disease, the development of other therapeutic approaches has been limited by the lack of a fundamental understanding of the disease's causes. This work opens the way to a concerted effort to understand the pathogenesis of Paget's disease of bone.

Kurihara N, Reddy SV, Menaa C, Anderson D, Roodman GD: Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype. The Journal of Clinical Investigation, 105(5):607-14. 2000.

New Neutrophil Types Identified. Neutrophils are whites cells that kill antibody-coated bacteria and fungi. An important neutrophil antibody binding receptor, Fc-gamma-recetor IIIb, is known to have two different forms or types. They are called NA1 and NA2 and they differ in their ability to bind to antibody-coated organisms. The genes encoding this receptor were studied in many different people and 5 new types were identified. Four of the new types are similar to the NA2 form of the gene and one was most similar to the NA1 form. These variations in Fc-gamma-receptor IIIb may help explain differences in responses of individuals to infections.

Matsuo K, Procter J, Stroncek D: Variations in genes encoding neutrophil antigens NA1 and NA2. Transfusion, 40(6): 645-53. 2000.

Exit Signs Help Traveling Neurons in the Brain. How does an exquisitely complex structure such as the human brain develop properly to form a fully functional unit? It has been shown that neurons – cells making up the brain and central nervous system – actually migrate. NIH-supported investigators

demonstrated that the final destination of migrating neurons is influenced by a protein called reelin, which is produced by a special type of cell at the outermost part of the developing brain. When the investigators measured the rate of neuronal migration, they found that reelin acts something like an exit sign, signaling cells to decrease their rate of migration. In addition, reelin added from outside by investigators actually stopped the migration of neurons in developing cerebral cortical neurons in rat embryos. The mechanism by which reelin exerts its actions appears to involve direct interaction with the protein integrin, as reelin was ineffective in directing the exit of neurons in preparations lacking this protein. This work has begun to identify molecular interactions necessary for establishing critical migratory events in the formation of the intricate folds and layers characteristic of the human brain. This is very important because these findings may aid in understanding the role of developmental factors such as reelin and other molecules in the etiology of several developmental disorders, such as autism and schizophrenia, which appear to involve erroneous development of neuronal connections.

Dulabon L, Olson EC, Taglienti MG, et al: Reelin binds a3ß1 integrin and inhibits neuronal migration. Neuron, 27(1): 33-44. 2000.

Why Prostate Cancer Homes to Bone. Prostate cancer has a high propensity for metastasizing and invading bone in the pelvis and vertebra of the lower back. Scientists now have convincing evidence that a substance in bone not only attracts the prostate cancer cells, but also stimulates them to become invasive. The factor is a protein called osteonectin that specifically enhances protein degradative enzyme activity. In their studies, the researchers showed that while osteonectin promotes the invasive ability of prostate and breast cancer cells, it is inactive against cancers that do not metastasize to bone. They have identified the receptor for osteonectin and have formed a partnership with a company to test whether levels of the receptor and osteonectin can be used as a diagnostic tool for determining the metastatic potential of prostate and breast cancer.

Jacob K, Webber M., Benayahu D, Kleinman HK: Osteonectin promotes prostate cancer cell migration and invasion: A possible mechanism for metastasis to bone. Cancer Research, 59(17):4453-57. 1999.

Genomic Approaches to Understanding Oral Cancer. Tobacco and alcohol use are known risk factors for oral cancer development, but the molecular mechanisms responsible for the disease are still poorly understood. Using a technique called Laser Capture Microdissection (LCM), NIH scientists 'captured' populations of normal, premalignant, and cancerous cells from tissue biopsies. They then used DNA microchip technology – which allows the analysis of hundreds of genes simultaneously – to study the genetic changes that correlate with the transition from healthy tissue to cancer. Through this research, the scientists have already identified several classes of genes that are altered in oral cancer cells. Additionally, the scientists have established a sub-project for head and neck cancers within the

NIH's Cancer Genome Anatomy Project (CGAP). CGAP's goal is to determine the profiles of genes active in cancers at various stages of development. Taken together, these research projects will allow scientists to learn more about the molecular basis of oral cancer, and to develop molecular markers of disease progression as well as new treatments.

Shillitoe EJ, May M, Leethanakul C, et al: Genome-wide analysis of oral cancer: early results from the cancer genome anatomy project. Oral Oncology, 36(1):8-16. 2000.

Leethanakul C, Patel V, Gillespie J, et al: Gene expression profiles in squamous cell carcinomas of the oral cavity: Use of laser capture microdissection for the construction and analysis of stage-specifi cDNA libraries. Oral Oncology, 36(5):474-83. 2000.

Leethanakul C, Patel V, Gillespie J, et al: Distinct pattern of expression of differentiation and growth-related genes in squamous cell carcinomas of the head and neck revealed by the use of laser capture microdissection and cDNA arrays. Oncogene, 19(28):3220-4. 2000.

Mutation of PAX9 is Associated with Oligodontia. Tooth agenesis, or the developmental absence of permanent teeth, is a common anomaly that affects about 20% of the population. Oligodontia is the absence of 6 or more teeth. A mutation in the PAX9 gene has been identified as the cause of oligodontia in a family in Texas. The mutation is inherited in an autosomal dominant fashion. Characterization of the role of PAX9 in tooth development will advance our understanding of the molecular mechanisms underlying tooth development and will lead to improved diagnosis, treatment and prevention.

Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI: Mutation of *PAX9* is associated with oligodontia. Nature Genetics, 24(1):18-19. 2000.

Impaired Osteoclast Function Produces Osteopetrosis. Osteopretosis involves the loss of metal contact in the bone, with the bone becoming spongy. When the bone becomes over-calcified, normal bone turnover does not occur; consequently, the bone doesn't reform and thus there is a loss of strength. This study involved examination of oseopretosis in mice, a syndrome that is also found in humans. The ability of osteoclasts, a type of cell found in bone, to break down bone is dependent on their ability to produce an acidic environment in the bone surrounding them. NIH-supported investigators have disrupted the function of a gene (Atp6i) that appears to be responsible for producing an osteoclast-specific proton pump, that is, a mechanism that has the ability to move hydrogen ions form inside the cell to outside the cell. Investigating the basis for the bone forming abnormalities revealed no change in osteoclast numbers or in their morphology. However, unlike wild type osteoclasts, Atp6i mutant osteoclasts failed to form lacunae or spaces after attachment to bone, indicative of impaired bone breakdown activity. These data and other results of this investigation provide important

information on a crucial step for the ability of osteoclasts to breakdown bone. The gene and gene product that allows the osteoclasts to produce extracellular acidification in bone are potential targets for therapeutic agents that prevent bone destruction.

Li YP, Chen W, Liang Y, Li E, Stashenko P: *Atp6I*-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nature Genetics, 23(4):447-51. 1999.

Cell Transplantation and Aging. Both scientific and lay communities are interested in the potential use of gene therapy and stem cell transplantation, as well as tissue transplantation, to combat diseases of aging. An alternative to tissue transplantation that appears to have great potential is the formation of functional tissue from cell transplants. Isolated bovine or human adrenocortical cells inserted into immunodeficient mice formed functional adrenal tissue that morphologically resembles normal adrenal gland. This approach may potentially be used for any organ, either to study its functional regeneration in a living organism as a function of age, or to therapeutically regenerate lost function (for example, by replacing defective genes in cells isolated from the patient before replacing them into the patient for tissue regeneration). This approach reduces the need for immunosuppressive therapies, and offers an alternative to controversial stem cell therapies in reducing disease.

Hornsby PJ: Cell transplantation and aging. Generations, 24:54-7, 2000.

Thomas M, Yang L, Hornsby PJ: Formation of functional tissue from transplanted adrenocortical cells expressing telomerase reverse transcriptase. Nature Biotechnology, 18(1):39-42. 2000.

Thomas M, Hornsby PJ: Transplantation of primary bovine adrenocortical cells into *scid* mice. Molecular and Cellular Endocrinology, 153(1-2):125-36. 1999.

Mutations in the BRI Gene Result in the Deposition of Amyloid and Consequent Dementia. Recent studies have shown that a number of dementias are associated with deposition of amyloid, including a recently identified mutation in the gene called "BRI" located on chromosome 13, causing the development of familial British Dementia (FBD). Patients have amyloid deposition associated with blood vessels, hippocampal plaques, and neurofibrillary tangles. A close variant of the familial British Dementia has recently been identified by U.S. scientists in collaboration with investigators in the U.K. and Denmark. The disease is found in a small population of Danish families and is also characterized by amyloid deposition. Like the British form, the Danish mutation also results in the formation of a longer than usual form of the BRI protein which is clipped to form an amyloid peptide, resulting in plaque formation. This study is significant because it shows another way in which mutations in the BRI gene can cause amyloid deposition and dementia and adds to the number of dementias caused by deposition of different forms of amyloid. Analysis of the ways in which this amyloid causes dementia will give insight into the mechanisms of degeneration in other amyloid-forming diseases such as Alzheimer's disease.

Vidal R, Revesz T, Rostagno A, et al: A decamer duplication in the 3' region of the *BRI* gene originates an amyloid peptide that is associated with dementia in a danish kindred. Proceedings of the National Academy of Sciences, 97(9): 4920-5. 2000.

Dietary Restriction Increases Neurotrophic Factor Production in the Brain and Thereby Protects Neurons. Simply reducing their caloric intake can increase the lifespan of rodents. Such caloric restriction (CR) reduces the development of age-related cancers, immune and neuroendocrine alterations, and motor dysfunction. Recent studies using animal models of neurodegenerative disorders provide the first evidence that CR can also increase resistance of neurons to age-related and disease-specific stresses. One possible mechanism for the beneficial effects of CR is that the mild metabolic stress associated with CR induces cells to produce proteins that increase cellular resistance to disease processes. This study demonstrates that CR increases production of one such protein, a neuronal survival factor, BDNF. It further demonstrates that BDNF signaling plays a central role in the neuroprotective effect of CR and suggests that CR may be an effective approach for reducing neuronal damage in neurodegenerative disorders.

Duan W, Lee J, Guo Z, Mattson MP: Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. Nature Neuroscience , 2001.

First Comparison of the Human Genome to a Complex Model Genome. The worm, *C. elegans*, is the first higher organism for which the DNA sequence for its complete genome, containing all of its genes and other genetic information, has been obtained. Using computer-based analysis techniques developed at the NIH, a comparison of the worm genome against the human genome sequence as it was known in late 1999 revealed a 50% nucleotide identity within the genes of the two organisms. In addition, of the genes known to be common to humans and rodents, about 44% are now also found in the worm, indicating that the worm provides a simplified but useful research model for interpreting the human genome.

Wheelan SJ, Boguski MS, Duret L, Makalowski W: Human and nematode orthologs--lessons from the analysis of 1800 human genes and the proteome of *Caenorhabditis elegans.* Gene, 238(1):163-70. 1999

Data from the Human Genome Project Reveals Widespread Shuffling of Human DNA by LINE Elements. LINE-1 elements represent a class of repetitive DNA present in up to 600,000 copies in the human genome and accounting for about 15% of human DNA. Each full-length LINE-1 element encodes an endonuclease and reverse transcriptase which it uses to replicate and move itself from place to place within human DNA. These genes are the most abundant genes in the human genome. It has been experimentally determined that LINE-1 elements are *capable* of carrying human DNA from one area of the genome to another, but whether this had actually *occurred* in the human

genome was another question. Using the human genome sequence data, it was possible to estimate the extent to which LINE-1 induced DNA migration had occurred. The researchers were able to show that approximately 1% of the human genome has in fact been shuffled by LINE-1 elements. This fraction is roughly equivalent to the portion of the human genome occupied by the exons of genes – the sections of DNA that actually contain the code for making proteins – and may represent a major factor for human genome re-modeling. This is a powerful example of how genomic information made available by the NIH has led to greater understanding of human genome.

Pickeral OK, Makalowski W, Boguski MS, Boeke JD: Frequent human genomic DNA transduction driven by LINE-1 retrotransposition. Genome Research, 10(4):411-15. 2000.

Clue to the Mechanism of Latency in Ocular Herpes Infection. Initial infection of the eye by HSV-1 leads to a lifelong latent infection of the ocular nerves. Periodically, the latent virus becomes reactivated causing recurrence of the disease, ocular pain, and scarring of the corneal tissues. While survival of HSV-1 in a latent state obviously requires survival of the host cells, infected cells normally undergo a process called programmed cell death, or apoptosis. Researchers recently discovered that a viral gene expressed during latency, the LAT gene, inhibits programmed cell death in infected nerve cells, thereby providing a host for the latent state. Understanding the mechanism of viral latency and reactivation is essential for the development of an effective therapeutic intervention in this painful and potentially blinding disease.

Perng GC, Jones C, Ciacci-Zanella J, et al: Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. Science, 287:1500-3. 2000.

New Images of a Major Lens Protein Give Insight into Protein Stability During Aging and Stress. Lens transparency requires high concentrations of protein within specialized lens fiber cells. Because fiber cell proteins are not removed or replaced during the life of an individual, the lens is especially susceptible to aging effects. As these proteins age, changes occur that cause them to aggregate into large, opaque structures that can interfere with vision. To maintain transparency, the lens must maintain the integrity of its proteins. In general, cells use a chaperone protein to protect other proteins when the cell is subjected to stress. In the lens, a-crystalline is believed to function as a chaperone. Recent studies have added new detail about the structure of this protein and how it acts to protect the lens from the potentially harmful effects of the aging process. These studies reveal a structure that is well adapted to the chaperone function and provide new insights into protecting against aging and cataract development.

Bova MP, Mchaourab HS, Han Y, Fung BKK: Subunit exchange of small heat shock proteins. The Journal of Biological Chemistry, 275(2):1035-42. 2000.

New Findings Help Explain How Patients with Glaucoma Lose Their Vision. Elevated interocular pressure has been associated with vision loss in glaucoma, but definitive evidence supporting a causal effect has not been demonstrated experimentally. Since all current treatments attempt to slow the progressive loss of neurons by reducing intraocular pressure, establishing this relationship would justify the continued use of these therapeutic strategies. Scientists now have evidence that increases in intraocular pressure have a profound effect on ganglion cell survival. Optic nerve fibers from retinal ganglion cells connect to neurons in a part of the brain called the lateral geniculate nucleus (LGN). Neurons from the LGN then relay this information to the visual cortex for processing. Using a primate model of glaucoma, scientists showed that even relatively moderate elevations of intraocular pressure cause loss of LGN neurons over an extended period of time. These data demonstrate that chronic elevation of intraocular pressure has a neurodegenerative effect on neurons critical for the integration and transmission of visual information.

Weber AJ, Chen H, Hubbard WC, Kaufman PL: Experimental glaucoma and cell size, density, and number in the primate lateral geniculate nucleus. Investigative Ophthalmology and Visual Science, 41(6):1370-9. 2000.

Yücel YH, Zhang Q, Gupta N, Kaufman PL, Weinreb RN: Loss of neurons in magnocellular and parvocellular layers of the lateral geniculate nucleus in glaucoma. Archives of Ophthalmology, 118(3):378-84. 2000.

Development of Myopia. More than 25% of the adult population of the United States is nearsighted (myopic). In the most serious cases, myopia can lead to retinal detachment, glaucoma, amblyopia, and vision loss. At present there is no definitive treatment or cure for myopia. Corrective lenses and corneal surgeries merely compensate for the condition, and these treatments do not affect the underlying predisposition to myopia. The cost each year to our society for eye examinations and corrective lenses for refractive error is enormous. Animal models have recently contributed two important insights to our understanding of the biological basis of myopia. First, images which are not focused on the retina guide the developing eye to correct for this defocus. Second, changes in the focus of images on the retina cause changes in eye growth directly. Through a cascade of signals from the retina to the sclera, the outer tunic of the eye, the axial length of the eye is changed. One class of retinal interneurons, amacrine cells, appear to mediate the regulatory mechanisms and intercellular signals which control eye growth in response to optical defocus. Additional research identifying the specific factors which control eye growth will have significant implications for preventing or treating myopia.

Fischer AJ, McGuire JJ, Schaffel F, Stell WK: Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. Nature, 2(8):706-12. 1999.

The Genetic Basis of Stargardt Macular Dystrophy. Stargardt macular dystrophy is associated with an inherited progressive loss in central vision. Affected individuals have decreased visual acuity and

progressive bilateral atrophy of the cone-rich fovea (a specialized region of the central retina or macula). Familial genetic studies have shown that this disease is associated with mutations in the ATP-binding cassette transporter (ABCR) gene. ABCR appears to function by removing photobleached retinal derivatives from cone cells. Recent gene expression studies have demonstrated that this gene is expressed in foveal and peripheral cone cells. Disease-linked ABCR mutations lead to an accumulation of retinal derivatives. The fovea may be particularly sensitive to accumulation of retinals, leading to preferential degeneration and loss of central vision as an early symptom of this disease.

Molday LL, Rabin AR, Molday RS: ABCR expression in foveal cone photoreceptors and its role in stargardt macular dystrophy. Nature Genetics, 25(3):257-8. 2000.

New Methods of Discovering Eye Tumor Genes. NIH researchers have a new weapon against ocular cancer – Laser Capture Microdissection. These scientists reported important findings using this highly specific technology of obtaining pure tumor cells from microscopic slides. Using this technique, the researchers have pinpointed the true location of cancer cells in retinal angiomas from von Hippel-Lindau disease, and subsequently identified the tumor genes in primary intraocular lymphoma, a potentially fatal eye tumor. These findings take the necessary steps towards better diagnosing and guiding the treatment of these diseases, which can cause blindness and even death.

Vortmeyer AO, Chan CC, Chew BY et al: Morphologic and genetic analysis of retinal angioma associated with massive gliosis in a patient with von hipel-lindau disease. Graefe's Archive for Clinical and Experimental Ophthalmology, 237: 513-7. 1999.

Shen DF, Fardeau C, Roberge FG, LeHoang P, Chan, CC: Rearrangement of immunoglobulin gene in metastatic waldenstrom macroglobulinemia to the vitreous. American Journal of Ophthalmology, 129(3):395-6. 2000.

Velez G, de Smet MD, Whitcup SM, Robinson M, Nussenblatt RB, Chan CC: Iris involvement in primary intraocular lymphoma: report of two cases and review of the literature. Survey of Ophthalmology, 44(6):518-26. 2000.

The Nature of Activity in the Superior Colliculus. The superior colliculus is an important structure in the generation of eye movements, particularly saccadic eye movements. How the superior colliculus shapes the neural commands to make saccadic eye movements is an area of active research. This work gives further evidence for the theory that a wave of activation travels in a specific direction across the superior colliculus during saccadic eye movements. Knowledge of the pathways used in making eye movements helps to localize the problem when disease causes abnormalities of eye movements.

Port NL, Sommer MA, Wurtz RH: Multielectrode evidence for spreading activity across the superior colliculus movement map. Journal of Neurophysiology, 84(1):344-57. 2000.

The RPE65 Gene and the Visual Cycle. The promoter region of the RPE65 gene was characterized. NIH scientists have demonstrated previously that this gene plays an important role in vitamin A metabolism in the retina. Critical DNA elements that play an important role in the regulation of RPE65 gene expression were also identified. Because RPE65 is essential for proper metabolic functioning in the retinal pigment epithelium and mutations in the RPE65 gene have been linked in human patients to retinal degenerations, it is of considerable importance to obtain a thorough understanding of the regulation of the RPE65 gene.

Boulanger A, Liu S, Henningsgaard A, Yu S , Redmond TM: The upstream region of the rpe65 gene confers retinal pigment epithelium-specific expression in vivo and in vitro and contains critical octamer and E-box binding sites. The Journal of Biological Chemistry, 275(40):31274-82. 2000.

Circadian Dependent Retinal Light Damage in Rats. A study was done to determine the relative susceptibility of rats to retinal light damage at different times of the day. Greater retinal light damage occurred in rats exposed at night than during the day, and a single, relatively short, intense light exposure caused a circadian dependent loss of photoreceptor cells. The light induced loss of photoreceptor cells was preceded by DNA fragmentation and by alterations in the gene functions in the retina and within the photoreceptors. These alterations at the onset of light exposure appear to be important in determining light damage susceptibility.

Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B: Circadian-dependent retinal light damage in rats. Investigative Ophthalmology and Visual Science, 41(12):3694-701. 2000.

Discovery of Novel Mutation in BLNK Provides New Insight into B Cell Development. Patients with B cell immunodeficiencies have recurrent, potentially life-threatening infections because they lack mature B cells. B cells produce antibodies, a key component of the immune system's defense against infectious agents. BLNK, also known as B cell linker protein, functions as a "molecular scaffold" bringing together, in the B cell, enzymes and the molecules on which they act. NIH-funded scientists recently identified a novel mutation in BLNK in a patient with B cell immunodeficiency. Further studies showed that mice with a similar mutation in BLNK, were also immunodeficient. This finding provides new insight into the critical role of BLNK in maintaining healthy immune responses. Other studies suggest that BLNK is present in other immune cells such as macrophages and thus suggest that BLNK and related proteins may be more broadly involved in immunoregulation than previously thought.

Minegishi Y, Rohreer J, Coustan-Smith E, et al: An essential role for BLNK in human B cell development. Science, 286(5446):1954-7. 1999.

Pappu R, Cheng AM, Li B, et al: Requirement for B cell linker protein (BLNK) in B cell development. Science, 286(5446):1949-54. 1999.

Bonilla FA, Fujita RM, Pivniouk VI, Chan AC, Geha RS: Adapter proteins SLP-76 and BLNK both are expressed by murine macrophages and are linked to signaling via Fc? receptors I and II/III. Proceedings of the National Academy of Sciences, 97(4):1725-30. 2000.

Diesel Exhaust Particles (DEPs) Induce Allergic Antibody. Allergic reactions involve a special class of antibody, called IgE. About 20% of Americans are considered "allergic" because they have a genetic tendency to produce IgE antibodies to certain proteins, such as ragweed pollen. NIH-funded scientists previously showed that DEPs, which are environmental pollutants, are not only toxic, but also strongly increase allergic airway inflammation and IgE production to ragweed allergens in people who are already allergic. Recently, these scientists discovered that exposure to DEPs also stimulates the production of IgE in response to newly-encountered proteins. These results indicate that air pollution due to diesel exhaust may have greater public health implications than previously appreciated.

Diaz-Sanchez D, Garcia MP, Wang M, Jyrala M, Saxon A: Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. Journal of Allergy and Clinical Immunology, 104(6):1183-8. 1999.

Discovery of New Pathway for Immune Recognition of Tuberculosis. Tuberculosis (TB) kills about 1.5 million people annually worldwide. The increasing incidence of multi-drug resistant TB is compounding the difficulties caused by the lack of effective agents to prevent and rapidly treat this disease. Recently, NIH-supported investigators, working with *Mycobacterium tuberculosis* (the organism that causes TB), determined the structure of a new class of mycobacterial glycolipids (molecules composed of fats and sugars) that are important for immune recognition of *Mycobacterium tuberculosis*. Knowledge about how the immune system recognizes *Mycobacterium tuberculosis* may lead to novel approaches for the development of a TB vaccine.

Moody DB, Ulrichs T, Muhlecker W, et al: CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. Nature, 404(6780):884-7. 2000.

Genetic Mutation Linked to Missing Immunoregulatory Molecule. Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare genetic disorder that results in uncontrolled activation of the immune system leading to death in infancy or early childhood. NIH-supported researchers found that a protein called perforin, which is part of the cell-destroying apparatus of killer T cells, is missing or inactive in FHL patients. These investigators propose that the absence of perforin leads to an accumulation of T cells and a loss of T cell control, resulting in a prolongation of T cell responses.

Knowledge of the molecular basis of FHL should lead to the development of better diagnostic approaches and new therapies for this disease. In addition, these insights may lead to new approaches to control undesired T cells responses in autoimmune diseases, transplantation, allergy, and asthma.

Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al: Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science, 286(5446):1957-9. 1999.

Mutation Inhibits Proper Immune System Functioning. Fas is a receptor on the surface of immune cells that consists of three protein chains (a trimer). Fas is involved in transmitting a signal for a cell to self-destruct through a process termed programmed cell death (PCD). PCD maintains the equilibrium of the immune cell population. Some Fas mutations prevent PCD resulting in a disease called autoimmune lymphoproliferative syndrome (ALPS). Recently, NIH investigators determined that the formation of defective Fas trimers is dependent on a region on each Fas chain called the pre-ligand assembly domain, which facilitates the binding of the three protein chains to form Fas. This finding overturns a previous theory on the cause of defective Fas trimers and, by providing insight into the mechanism of PCD, should facilitate the development of approaches to treating ALPS and other lymphoproliferative disorders.

Siegel RM, Frederiksen JK, Zacharias DA, et al: Fas pre-association required for apoptosis signaling and dominant inhibition by pathogenic mutations. Science, 288(5475):2354-7. 2000.

Degradation of Defective Proteins Makes Antigens Available to Stimulate Immune Responses. The antigens that trigger the human immune system in response to pathogens are pieces of protein from the pathogen. In some cases, these proteins are made inside the infected cell, subsequent to entry of the pathogen. NIH scientists and colleagues discovered that a significant fraction of antigens derive from defective proteins that are destroyed soon after they are synthesized. The fact that a significant number of antigens derive from defective newly synthesized proteins, rather than from the degradation of intact functional proteins, helps explain the variability in the immune response to infection and how the immune system reacts early and continuously in the process of infection.

Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR: Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature, 404(6779):770-4. 2000.

Identification of Protein Responsible for Persistence of Kaposi's Sarcoma-Associated Herpesvirus. Herpesviruses are a family of viruses that cause a variety of diseases including cold sores and a sexually transmitted disease (herpes simplex virus I and II), chickenpox (varicella-zoster), and Burkitt's lymphoma (Epstein-Barr virus). Human herpesvirus 8 has been implicated in the development

of several immune system diseases and Kaposi's sarcoma, a connective tissue cancer characterized by tumors below the skin. Although cells from these lesions are infected with this Kaposi's sarcoma-associated herpesvirus (KSHV), it is not known how KSHV persists in cells and leads to malignancy. Recently, investigators from the new NIH Vaccine Research Center demonstrated that a specific viral protein, called latency associated nuclear antigen, can inhibit cell death after KSHV infection by inhibiting a protein called p53. p53 prevents mutated cells from replicating and induces cell-death in malignant cells. The elucidation of this mechanism of KSHV persistence in cells is an initial step in the design of approaches to block the development of KSHV-induced malignancies.

Friborg J Jr, Kong W, Hottiger MO, Nabel GJ: p53 inhibition by the LANA protein of KSHV protects against cell death. Nature, 402(6764):889-93. 1999.

Discovering the Genetic Basis of Hearing Loss. Hearing loss has enormous impact on both individuals and populations. U.S. scientists and counterparts in Israel and Palestine are collaborating on mapping and cloning the genes responsible for different types of inherited deafness (progressive and early-onset) in families from Israel and Palestine, where the incidence of preverbal deafness is among the highest in the world. They have identified a new gene related to deafness and critical mutations in another deafness gene. These studies may lead to treatment of both inherited and environmentally caused hearing loss.

Vahava O, Morell R, Lynch ED, et al: Mutation in the transcription factor *POU4F3* associated with inherited progressive hearing loss in humans. Science, 279(5358):1950-4. 1998.

Sobe T, Erlich P, Berry A, et al: High frequency of the deafness-associated 167delT mutation in the connexin 26(GJB2) gene in israeli ashkenazim. American Journal of Medical Genetics, 86(5):499-500. 1999.

Longevity Genes. A cooperative research effort involving U.S. and Russian scientists used natural populations of Drosophila to identify genetic loci that influence longevity. A set of 500 recombinant inbred (RI) strains were generated, backcrossed to the parental lines, and evidence for loci for longevity scored. The short life cycle of the insects allowed work to proceed relatively rapidly. The investigators were also able to investigate gene/environment interactions by changing the environment of the flies from optimal conditions to stressful conditions then measuring changes in longevity. The fine scale mapping obtained from this study may suggest longevity candidate genes in humans for future research.

Vieira C, Pasyukova EG, Zeng ZB, et al: Genotype-environment interaction for quantitative trait loci affecting lifespan in *Drosophila melanogaster*. Genetics, 154(1):213-27. 2000.

Pasyukova EG, Vieira C, Mackay TFC: Deficiency mapping of quantitative trait loci affecting longevity in *Drosophila melanogaster*. Genetics, 156(3):1129-46. 2000.

Working With the Building Blocks of Life. Genes provide the blueprint for formation of a myriad of proteins. Yet how does a part of the building machinery, called transfer ribonucleic acid (tRNA) function as the key molecule responsible for building this diversity of proteins from a common set of 20 amino acids? An international team of researchers from the United States and Croatia have provided significant insights into different structure and assignments of tRNAs. This research provides the basis for the design of specific blockers of protein building that may ultimately be applied in a wide variety of human and animal disorders and diseases.

Rokov J, Söll D, Weygand-Durasevic I: Maize mitochondrial seryl-tRNA synthetase recognizes *Escherichia coli* tRNA(Ser) in vivo and in vitro. Plant Molecular Biology, 38(3):497-502. 1998.

Lenhard B, Praetorius-Ibba M, Filipic S, Söll D, Weygand-Durasevic I: C-terminal truncation of yeast SerRS is toxic for *Saccharomyces cerevisiae* due to altered mechanism of substrate recognition. FEBS Letters, 439(3):235-40. 1998.

Lenhard B, Orellana B, Ibba M, Weygand-Durasevic I: tRNA recognition and evolution of determinants in seryl-tRNA synthesis. Nucleic Acids Research, 27(3):721-9. 1999.

Elucidating the Role of Growth Factors in Embryonic Development. Embryonic growth is regulated in part by an array of growth factors, some identified, others yet to be discovered. One growth factor, acrogranin, was studied to determine its role in early embryonic growth prior to implantation in the mammalian uterus. Using different concentrations of acrogranin *in vitro* with mouse embryos at early stages of development, findings suggest that acrogranin plays a role in the stimulation of the eight-cell stage embryo to the blastocyst stage and that it increases the numbers of epithelial cells involved in embryonic implantation in the uterus. These findings increase the knowledge base on mammalian fertility and embryonic development.

Diaz-Cueto L, Stein P, Jacobs A, Schultz RM, Gerton GL: Modulation of mouse preimplantation embryo development by acrogranin (epithelin/granulin precursor). Developmental Biology, 217(2):406-18. 2000.

Fruit Fly Genome Sequence will Provide Further Insights to Cancer and Aging. *Drosophila melanogaster,* more commonly known as the fruit fly, has been a powerful model system in biology since the early the 20th century. *Drosophila* studies have played a pivotal role in research ranging from aging and cancer to learning and memory. The sequence of the euchromatic portion of the genome of *Drosophila* is close to completion. The bulk sequencing was carried out by scientists at Celera Genomics, the University of California at Berkeley, and the Baylor College of Medicine. The sequence data are available through GenBank, and the annotation is available through the GenBank and FlyBase

databases. The combination of whole genome shotgun sequencing with a mapped BAC-by-BAC approach was effective, yielding ~120 Mb of data containing ~2400 gaps. NIH continues to fund the Berkeley and Baylor efforts to close the remaining gaps, to elucidate complex repeat regions, and to ensure the finished sequence meets quality standards for finished data. Approximately 400 gaps remain at this time. NIH also supports the development of methods for sequencing the heterochromatic portions of the *Drosophila* genome in a continuing project at the Salk Institute for Biological Studies.

Adams MD, Celniker SE, Holt RA, et al: The genome sequence of *Drosophila melanogaster*. Science, 287(5461):2185-95. 2000.

Myers EW, Sutton GG, Delcher AL, et al: A whole-genome assembly of *Drosophila*. Science, 287(5461):2196-204. 2000.

Genetic Mutation Causes Common Defect in Early Development of Human Forebrain. An international team led by scientists at the NIH located one of the genes that can cause holoprosencephaly, the most common structural defect of the developing forebrain in humans. It results in varying degrees of mental retardation. The finding suggests that the gene, TG-interacting factor (TGIF), plays an important role in the brain's separating into left and right hemispheres during fetal development. The TGIF gene is the fourth found in humans to be involved in HPE.

Gripp KW, Wotton D, Edwards MC, et al.: Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. Nature Genetics, 25(2):205-8. 2000.

Cocaine High Related to How and How Quickly the Drug Reaches the Brain. Epidemiological studies suggest that smoked and intravenous (iv) cocaine have greater abuse liability than intranasal cocaine. In some studies, subjects have also reported that smoked cocaine produces a greater "high" than cocaine taken intravenously. To examine the biological basis for these differences, researchers used positron emission tomography (PET) to compare the ability of cocaine to block dopamine transporters (DAT) in the brain. The DAT is the major target of cocaine and is believed to mediate the pleasurable effects of cocaine. Researchers found that equivalent amounts of cocaine given either intranasally, iv, or smoked, blocked similar numbers of DAT in each of the subjects. However, subjects who smoked cocaine reported a greater "high" than those who took cocaine intranasally. Those who received cocaine iv were intermediate in response, but most similar to those who smoked the drug. These findings suggest that the difference in reported "high" are not due to the level of DAT blockade. Rather, the cocaine "high" is more likely influenced by how fast the drug reaches the brain. Cocaine levels reached their peak amounts within 2 minutes for those who smoked, about 3 minutes for iv and 15 minutes for the intranasal group, which parallels the reported "high" from the drug. Understanding

how a drug produces its subjective effects will aid investigators in their efforts to develop more effective treatments for addiction.

Volkow ND, Wang GJ, Fischman MW, et al: Effects of route of administration on cocaine induced dopamine transporter blockade in the human brain. Life Sciences, 67(12):1507-15. 2000.

Marijuana Ingredient May Promote Tumor Growth. Researchers found that THC, the major psychoactive component of marijuana, may promote tumor growth. Intermittent doses of THC promoted the growth of tumors in mice injected with cancer cells by increasing the levels of specific cytokines (inter-cellular messengers) that signal the suppression of an immune response. The investigators also found that giving the mice a specific antagonist (neutralizer) of the cannabinoid receptor blocked the effect of THC. These findings suggest that THC promotes tumor growth by inhibiting the body's anti-tumor immune response. They also suggest that regular use of marijuana may increase the risk of respiratory tract cancer. More studies are needed to better understand the implications of these results.

Zhu LX, Sharma S, Stolina M, Gardner B, Roth MD, Tashkin DP, Dubinett SM: ?-9-Tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. Journal of Immunology, 165(1):373-80. 2000.

Moderate Weight Loss OK for Overweight Moms Who Breast Feed. Weight gained during pregnancy may contribute to obesity later in life, which can lead to serious health problems; however, new mothers also need sufficient fat reserves to produce an adequate amount of milk for their infants. Researchers have demonstrated that overweight mothers who breast-feed may safely lose weight through a sensible diet and exercise program without fear of affecting their milk production and harming their infants.

Lovelady CA, Garner KE, Moreno KL, Williams JP: The effect of weight loss in overweight, lactating women on the growth of their infants. The New England Journal of Medicine, 342(7):449-53. 2000.

Phospholamban Affects Contractility of the Heart. It is well known that heart muscle contractions increase in response to increased frequency of specific stimuli, but the underlying mechanism is not understood. In this study, phospholamban, a specific regulator of the calcium transport mechanism in the heart muscle, was evaluated for its involvement in cardiac contractility. Heart muscle tissue from mice with genetically deleted phospholamban failed to respond in the expected manner, while heart tissue from wild-type responded as predicted. The researchers concluded that they have identified

phospholamban as a major determinant of the unknown contributors to the cardiac force-frequency relationship.

Bluhm WF, Kranisa EG, Dillmann WH, Meyer M: Phospholamban: a major determinant of the cardiac force-frequency relationship. American Journal of Physiology-Heart and Circulatory Physiology, 278(1):H249-H255. 2000.

Understanding Narcolepsy. Narcolepsy is a serious brain disorder that affects sleep in a dramatic way. Symptoms include extreme daytime sleepiness, a frightening inability to move shortly after awakening or dozing off, and sudden episodes of muscle weakness called cataplexy. In 1999 scientists discovered a defective gene that causes narcolepsy in dogs, one of the few animals that exhibit this disorder. The gene carries the instructions for making a receptor by which nerve cells respond to a brain signaling molecule called hypocretin. Guided by the animal findings, clinical studies in people, reported in 2000, now show that most people with narcolepsy have abnormally low levels of the hypocretin signal itself in the brain because the nerve cells that normally produce this molecule either die or stop producing this substance. Supplying hypocretin, or drugs that mimic its actions, may help prevent or treat the disease.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E: Hypocretin (orexin) deficiency in human narcolepsy. The Lancet, 355(9197):39-40. 2000.

Peyron C, Faraco J, Rogers W, et al: A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nature Medicine, 6(9):991-6. 2000.

Pain Memories. Pain sensation is obviously useful to help protect the body from damage. However, a painful injury often leads to abnormally persistent pain or to hypersensitivity in which even light touch can cause pain. Scientists have now discovered that chemical signals, called extracellular signal-regulated protein kinases (ERKs), may play a role in long lasting pain conditions. ERKs have been previously implicated in the sequence of events that lays down memories, so persistent pain states may be, in effect, "painful memories" within the spinal cord. In animal experiments blocking the activity of ERKs reduced the formation of pain hypersensitivity. Better understanding of what causes persistent pain states may lead to ways to better treat, or even prevent, pain hypersensitivity.

Ji RR, Baba H, Brenner GJ, Woolf CJ: Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nature Neuroscience, 2:1114-9. 1999.

Obesity and Malalignment Correlate With Knee Osteoarthritis. Osteoarthritis (OA) is the most common disease of joints. As the number of older people in our population continues to grow, OA can

be expected to affect more of the American public. Scientific opportunities to prevent OA are increasing as more modifiable risk factors are being identified. Obesity is most strongly linked to osteoarthritis of the knee, as opposed to the hand or the hip. Metabolic factors do not appear to be implicated because studies have failed to find an association between body fat distribution, diabetes, cholesterol level, uric acid level, or blood pressure with knee OA. Researchers studied the mechanical factors underlying the effect of obesity on the knee joint and the implication for osteoarthritis of the knee. Alignment may influence risk of progression and responsiveness to treatment among obese subjects.

Sharma L, Lou C, Cahue S, Dunlop DD: The mechanism of the effect of obesity in knee osteoarthritis: the mediating role of malalignment. Arthritis and Rheumatism, 43(3):568-75. 2000.

Exercise Results in Metabolic Shifts Lasting Several Days. Muscle use requires physiological adjustments for the body to meet many metabolic and cardiovascular demands during activity and restorative demands during subsequent rest. These adjustments vary according to the kind of exercise – endurance or resistance. Researchers recently evaluated the multiday time course of protein synthesis and glucose uptake in muscle following resistance exercise. This is the first study to provide a detailed time course showing the activation of several metabolic pathways following resistance exercise. Comparing the effects of resistance and endurance exercise will show how muscle adapts to the different demands placed on it and enable researchers to better understand how muscles and the body balance the need for different types of force and power with energy supply.

Hernandez JM, Fedele MJ, Farrell PA: Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. Journal of Applied Physiology, 88(3):1142-9. 2000.

Molecular Mechanisms of Calcium Influx. Certain types of cells need high levels of calcium to function properly. When these cells sense that more calcium is needed, they activate a cell membrane protein called an ion channel. The channel functions as a 'door,' which opens to let calcium flow in from the outside. This process is called calcium influx. Researchers have discovered that a newly identified family of proteins called Trp may be involved in forming the channel or may control its opening and closing. Learning how this process works and identifying which molecules are involved in it will be important for developing new therapies based on controlling calcium influx. Such therapies may be helpful for a wide variety of diseases and disorders since almost every type of tissue has cells that perform calcium influx.

Liu X, Wang W, Lockwich T, et al: Trp1, a candidate for the store-operated Ca^{2+} influx mechanism in salivary gland cells. The Journal of Biological Chemistry, 275(5):3403-13. (2000).

Lockwich TP, Liu X, Singh BB, Jadloweic J, Weiland S, Ambudkar IS: Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. The Journal of Biological Chemistry, 275(16):11934-42. 2000.

Changes in Physiological Response Characteristics of Cortical Neurons in Aging Primate Brain. Studies of visual perception in humans have characterized age-related changes, which include decreases in visual acuity, binocular summation, contrast sensitivity, motion sensitivity and color perception. This study goes beyond the eye to explore age-related changes within the central nervous system. Although a previous anatomical study found that the first brain area to receive visual signals (the dorsal lateral geniculate nucleus of the thalamus) is normal in older monkeys, this study is the first one to demonstrate differences at the next higher level of processing in the primary visual cortex (striate cortex or V1). Neurons in this cortex respond to different types of stimulation. Some neurons are sensitive to the orientation and direction of the light stimuli. With aging, the selectivity of neurons that respond to changes in orientation and direction was found to decrease, and the neurons were found to have increased responsiveness to all orientations and directions as well as an increase in spontaneous activity. These data suggest that neurons in the brain that are important to help an individual visually relate to the local environment lose the ability to discriminate with age. This loss may cause people to see less well as they age.

Schmolesky MT, Wang Y, Pu M, Leventhal AG: Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. Nature Neuroscience, 3(4):384-90. 2000.

Changes in Diapered and Nondiapered Infant Skin. Unlike the skin of adults that is exposed to prolonged moisture, the skin of newborn infants does not exhibit breakdown even though it is continuously exposed to water in the mother's uterus. Following birth, skin changes occur such that skin breakdown (diaper rash) is seen. Investigators evaluated the skin moisture and acid level of 31 newborn infants over the first month of life in both diapered and non-diapered skin areas and compared the infants' skin properties to adult skin. During the first 14 days after birth the infants' skin moisture increased, and these increases were more pronounced in diapered skin areas. The skin became more acidic during the first 7 days after birth, especially in the non-diapered areas. Adult skin was different than the neonates' and showed no changes over time. Better understanding of the skin changes may be useful in designing care regimens to prevent breakdown that could be a source of discomfort and infection.

Visscher MO, Chatterjee R, Munson KA, Bare DE, Hoath SB: Changes in diapered and nondiapered infant skin over the first month of life. Pediatric Dermatology, 17(1):45-51. 2000.

Structural Characterization of Tear Components. The external surface of the eye is covered by a thin tear film that lubricates and protects it from the external environment. Human tears that form this film consist of several proteins that are essential for its maintenance and proper function. Three major proteins are packaged and secreted together by the lacrimal gland that produces tears. One of these proteins, lipocalin, strongly interacts with the other two proteins, as well as with lipids. Scientists are uncovering those structural features of this molecule that confer its capacity to interact with other tear components. Knowledge of the structural basis for tear formation will lead to better strategies to treat dry eye, an often-debilitating condition affecting millions of people in the United States.

Gasymov OK, Abduragimov AR, Yusifov TN, Glasgow BJ: Interaction of tear lipocalin with lysozyme and lactoferrin. Biochemical and Biophysical Research Communications, 265(2):322-5. 1999.

Gasymov OK, Abduragimov AR, Yusifov TN, Glasgow BJ: Resolution of ligand positions by site-directed tryptophan fluorescence in tear lipocalin. Protein Science, 9(2):325-31. 2000.

Plasma Membrane Cholesterol Release Modulates the Activation of Mammalian Sperm. The activation of sperm is mediated through multiple biochemical pathways. One such pathway includes a time-dependent increase in protein phosphorylation that is dependent on the presence of albumin, calcium, and bicarbonate. This study further refines the role of this pathway by demonstrating that cholesterol release from the sperm plasma membrane is associated with the activation of a transmembrane signal transduction pathway which leads to the functional maturation of sperm.

Visconti PE, Ning X, Fornes MW, et al: Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. Developmental Biology, 214(2):429-43. 1999.

New Insights into the Natural History of Hepatitis C Virus Infection in Injection Drug Users. A majority of injection drug users (IDUs) are infected with hepatitis C virus (HCV). In most people with HCV infection the virus persists, resulting in a chronic infection. After 10-30 years persons with persistent HCV infection may remain asymptomatic or may develop end stage liver disease (ESLD). Estimates of ESLD vary widely and there is little consensus on disease co-factors, such as HIV and hepatitis B infection, or predictors of disease progression. New data from a community-based cohort of IDUs demonstrate that the majority of those infected with HCV had persistent viral particles in the blood without clinically demonstrable liver disease. The incidence of ESLD was 3.1 cases per 1000 person years; persons 38 years and older had a fourfold increase in incidence of ESLD, and those reporting ingestion of three or more drinks daily were 3.6 times more likely to have ESLD. The relative incidence of ESLD also increased with more frequent injection drug use. No increased risk of ESLD was observed for HIV-infected participants, for African Americans, or for those infected with hepatitis

B, although clearance of HCV was less likely to occur among African-Americans and among IDUs infected with HIV. In this cohort of IDUs, only 1 of 1667 persons infected with HCV received therapy for HCV.

Thomas DL, Astemborski J, Rai RM, et al: The natural history of hepatitis C virus infection: host, viral, and evvironmental factors. The Journal of the American Medical Association, 284(4):450-6. 2000.

Home Culture, Health Status Influence Infant Mortality Among U.S. Born Puerto Ricans. The Puerto Rican Maternal and Infant Health Study is the first survey to examine how different elements of immigrant cultures might influence health outcomes. The study compared native-born Puerto Ricans and U.S.-born Puerto Rican women. Researchers found that the longer native Puerto Rican women lived in the U.S., the higher the risk of infant mortality for their newborns, confirming the notion that immigrant culture can be protective. However, when comparing recent immigrants from Puerto Rico with women who still reside in the island, researchers found that infant mortality is substantially lower among the new U.S. immigrants, confirming the notion of "selectivity" among immigrants. This concept basically suggests that healthier women are more likely to immigrate. With such findings, researchers can start identifying factors found in immigrant cultures that are most important in maintaining or improving health outcomes.

Landale NS, Oropesa RS, Gorman BK: Migration and infant death: assimilation or selective migration among Puerto Ricans? American Sociological Review, 65:888-909. 2000

Zidovudine Does Not Increase Adverse Pregnancy Outcomes. To help decrease the chance that a mother may pass HIV to her unborn child, HIV-infected pregnant women often take the drug zidovudine, an antiretroviral therapy. Scientists found that compared to women who are not infected with HIV, HIV-infected women treated with zidovudine do not have an increased chance of an adverse pregnancy outcome (APO) such as preterm birth, low birth weight, and intrauterine growth retardation. Given that African American women are disproportionately affected by HIV and are at higher risk for APOs, this finding is particularly important since it suggests that zidovudine, a widely used treatment, does not put this population at an even greater risk of an APO.

Lambert JS, Watts DH, Mofenson L, et al: Risk factors for preterm birth, low birth weight, and intrauterine growth retardation in infants born to HIV-infected pregnant women receiving zidovudine. AIDS, 14:1398-9. 2000.

Women's Fertile Days are Highly Unpredictable. Women who are not using reliable contraception may sometimes rely on having intercourse during a "safe" time of their cycle. While the average woman is fertile for only a few days in each cycle, the actual timing of those fertile days is hard to predict.

Recent evidence from the NIH Early Pregnancy Study suggests women have very few perfectly safe days. The day of ovulation varies widely even in healthy women in their prime reproductive years, and only the first two or three days of the cycle can be considered completely safe. Women who are late in their cycle, expecting their next menses to begin any day, are still at some small risk of pregnancy with intercourse.

Wilcox AJ, Dunson D,Baird DD: The timing of the "fertile window" in the menstrual cycle: day-specific estimates from a prospective study. British Medical Journal, 321(7271):1259-62. 2000.

Psychosocial and Socioeconomic Factors Determine Who Stays in Long-Term Studies.
Researchers who investigate study participants over time worry that their results will be biased because individuals who remain active participants may be characteristically different from those who drop out; that is, the internal validity of a study may be threatened because individuals who drop out may somehow differ from those who remain. It has been hypothesized that individuals with the most severe pain and disability may be the least able to participate, or individuals may drop out when their symptoms flare. Researchers followed almost 1,000 patients with rheumatoid arthritis for 10 years. The results suggest that psychosocial and socioeconomic factors are more important determinants of continued participation in long-term research studies than are most clinical disease characteristics. Long-term studies of chronic diseases will need to oversample patients who do not have positive psychosocial and socioeconomic characteristics to compensate for the increased dropout rate of the former.

Reisine S, Fifield J, Winkelman DK: Characteristics of rheumatoid arthritis patients: who participates in long-term research and who drops out? Arthritis Care and Research, 13(1):3-10. 2000.

Presence of Rheumatoid Arthritis is Predictive of Development of Future Comorbidities.
Arthritis is the most common chronic disease in the United States. It is well recognized that disability escalates among people with arthritis who are burdened with additional chronic conditions. Recent studies have shown that disability does not rise linearly with the number of chronic conditions, but nearly exponentially. That is, the impact of multiple chronic diseases is not additive; it is multiplicative. In addition, several investigators have reported that people with arthritis are significantly more likely to experience comorbidity (the presence of more than one medical problem) compared to age- and sex-matched peers. In a 10-year followup study of population-based data, researchers found that patients with rheumatoid arthritis had a higher likelihood of developing congestive heart failure, chronic pulmonary disease, dementia, and peptic ulcer disease. Much of the excess in peptic ulcer and renal disease comorbidity observed is likely attributable to the use of nonsteroidal anti-inflammatory drugs. As the U.S. population ages, people with rheumatoid arthritis may represent a subgroup with disproportionate increases in disability and mortality.

Gabriel SE, Crowson CS, O'Fallon WM: Comorbidity in arthritis. Journal of Rheumatology, 26:2475-9. 1999.

New Insights Into the Ethical Conduct of Clinical Research. Biomedical or behavioral research involving human subjects, also known as clinical research, is critical to understanding human health and illness and finding new methods of preventing, detecting and treating disease. Nonetheless, clinical research must be designed and conducted in a way that avoids exploitation and is respectful of the rights and welfare of research participants. Numerous codes of ethics and regulations have been developed over recent decades to guide the ethical conduct of clinical research. Now NIH investigators have developed a systematic framework for designing and evaluating the ethics of clinical research. The framework of seven elements extrapolates from and builds on previous guidelines, providing a useful tool for clinical investigators, NIH Institute Review Board members, and others.

Emanuel EJ, Wendler D, Grady C: What makes clinical research ethical? The Journal of the American Medical Association, 283(20):2701-11. 2000.

Quality of AMI Care for Medicare HMO Enrollees Equal or Better than for Fee-for-Service Medicare Recipients. Concerns that the health care of elderly patients may be affected by HMO gatekeepers delaying newly developed treatment for enrollees and/or restricting access in emergency situations appear unfounded, at least in one particular setting. NIH supported research indicates no difference in care outcomes between elderly acute myocardial infarction (AMI) Medicare HMO and standard Medicare (fee-for-service or FFS) patients in 20 Minnesota community hospitals. In fact, three indicators of care adequacy for AMI were better among HMO patients. Emergency transportation, especially when the symptoms appeared at night, was used more often by HMO patients. Further, aspirin therapy and ß-blocker therapy were more likely for HMO than FFS patients. However, when controlling for age of physician, the differences in use of ß-blocker therapy ceased to be significant. All other indicators of quality were identical for the two groups: involvement of a cardiologist; treatment delay; time to electrocardiogram; use of thrombolytic agents; and time from hospital arrival to initiation of thrombolytic therapy.

Soumerai SB, McLaughlin TJ, Gurwitz JH, et al: Timeliness and quality of care for elderly patients with acute myocardial infarction under health maintenance organization vs. fee-for-service insurance. Archives of Internal Medicine, 159(17):2013-20. 1999.

Cholesterol May be a Modifiable Environmental Risk Factor for Alzheimer's Disease. The APOE-_4 allele is a risk factor for the development of Alzheimer's disease (AD) in most populations; however, its role in African Americans is not clear. APOE also plays a role in cholesterol transport, and studies have suggested an interaction among serum cholesterol, APOE genotype, and AD. In this

study, the interaction between total serum cholesterol and APOE genotype was evaluated in a population-based cohort of older African Americans. Increasing total cholesterol was associated with increased AD risk in the group with no APOE-_4 alleles, but total cholesterol was not associated with increased AD risk in the group with one or two _4 alleles. Although this study was cross-sectional and needs to be followed up by longitudinal studies, it does suggest that cholesterol may be a potentially modifiable risk factor for AD in some people. Interestingly, this is consistent with recent animal studies indicating that a high
cholesterol diet may increase the levels of beta amyloid plaques in brains of transgenic mouse models of AD.

Evans RM, Emsley CL, Gao S, Sahota A, Hall KS, Farlow MR, Hendrie H: Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: a population-based study of african americans. Neurology, 54(1):240-2. 2000.

Parkinsonism and Cognitive Decline in Alzheimer's Patients. Both parkinsonism (i.e., slowness of movement, rigidity, tremor, and gait disorder/postural reflex impairment) and Alzheimer's disease (AD) result from age-related neurodegenerative processes in which nerve cells are lost, levels of specific brain chemicals are altered, and/or abnormal deposits are found. Few studies have investigated changes over time to determine if the cognitive decline in the AD patients had any relationship to parkinsonism. A large cohort of AD patients was followed over a four-year time period. At the baseline, higher levels of parkinsonism were associated with lower levels of cognitive function. Furthermore, a more rapid cognitive decline was seen in those patients who had the higher levels of parkinsonism at baseline. Therefore, the variation in rate of cognitive decline in the AD patients could be predicted from knowledge of the level of parkinsonism alone. Whether or not this association can be traced to a common underlying mechanism of neurodegeneration between the two conditions, AD and parkinsonism, will require further study.

Wilson RS, Bennett DA, Gilley DW, Beckett LA, Schneider JA, Evans DA: Progression of parkinsonism and loss of cognitive function in alzheimer's disease. Archives of Neurology, 57(6):855-60. 2000.

The Sex Steroid, Testosterone, Modifies Working Memory in Elderly Men. Links between sex steroids, such as estrogen and testosterone, and cognitive function have been noted for over a decade. In this study, testosterone supplementation for one month in older men reversed declines in working memory, a type of memory in which the individual needs to hold information temporarily for later updating and/or retrieval. A similar enhancement of working memory was not found in older women supplemented with estrogen. These findings suggest that short-term administration of the sex steroid testosterone is capable of modulating a specific kind of memory in men and that short-term testosterone supplementation in older men can reverse age-associated declines in working memory. While further

exploration is warranted, and the long-term effects of testosterone supplementation on memory are as yet unknown, these data point to the need to evaluate testosterone levels in elderly men with short term memory or attention problems that could be linked to working memory decline. Future research may lead to the development of testosterone as a therapeutic in men with age-related memory deficits.

Janowsky JS, Chavez B, Orwoll E: Sex steroids modify working memory. Journal of Cognitive Neuroscience, 12(3): 407-14. 2000.

Attending to Cultural Language Improves Assessment of Symptoms. The words currently used to describe the symptom of breathlessness are derived from samples of primarily white subjects, and do not reflect the impact of culture and the language of symptom perception. In a laboratory setting during an asthma attack, African Americans described their breathlessness differently from Caucasians, with the African Americans using more upper airway descriptors. Educating health professionals about the unique ethnic language of breathlessness may prevent the under treatment of acute asthma episodes in African Americans.

Hardie GE, Janson S, Gold WM, Carrieri-Kohlman V, Boushey HA: Word descriptors used by african-american and white asthma patients during induced bronchoconstriction. Chest, 117(4):935-43. 2000.

Information for Cancer Patients. Short hospital stays are shifting cancer care to patients and their families. After undergoing surgery, newly diagnosed cancer patients need various types of information to make a successful transition from hospital to home. A recent study looked at the records kept by advanced practice nurses of their discussions with 148 cancer patients in the hospital and during follow-up home visits and telephone consultations. Nurses dispensed information on postoperative self-care, symptom management, the illness experience, psychological responses, coordination of follow-up care, and community resources. Teaching varied by cancer site and ranged from concrete instruction about wounds to complex interpretation of treatment options. Before discharge, patients could not take in or anticipate all the information they would need. Experienced nurses using a cost-effective combination of home and telephone contacts may be able to provide necessary ongoing support and information.

Hughes LC, Hodgson NA, Muller P, Robinson LA, McCorkle R: Information needs of elderly postsurgical cancer patients during the transition from hospital to home. Journal of Nursing Scholarship, 32(1):25-30. 2000.

Understanding the Experience of Sudden Cardiac Arrest. Every year approximately 500,000 Americans experience sudden death and of these, 29% survive the event and have an internal cardioverter defibrillator (ICD) placed. Nurse investigators used a grounded theory approach to identify areas of concern to individuals and family members of those who had an ICD placed. Over the

12 months of the study, seven areas of concern were identified. "Deal with ICD shocks" was considered to be the most difficult challenge. Patients and families also identified helpful strategies that would make the process of adjusting to the ICD more positive such as dealing with memory loss, driving restrictions, ICD shocks and general issues. Findings from this study provide the framework for designing more effective nursing interventions for this population.

Dougherty CM, Benoliel JQ, Bellin C: Domains of nursing intervention after sudden cardiac arrest and automatic internal cardioverter defibrillator implantation. Heart and Lung, 29(2):79-86. 2000.

Paths from Miscarriage to Depression. Nurse investigators tested a promising model for predicting depression after miscarriage. They hypothesized links between "contextual" variables (such as maternal age, weeks of gestation, number of miscarriages, and family income), "interceding" variables (such as social support and emotional strength) and three aspects of a woman's unfolding experience of miscarriage: her appraisal of its significance, her efforts to cope, and her depression. Applying data from 174 women whose pregnancies miscarried before 20 weeks, the researchers found that their model accounted for 63% of the women's depressive symptoms at 4 months and 54% at 1 year. Their work indicated that a woman's appraisal of her miscarriage was the best predictor of depression, along with her coping style, social support, emotional strength, family income, and subsequent pregnant status. Women who coped passively, engaging in self-blame or wishful thinking, were likely to be depressed, as were those with lower incomes. Context variables other than income showed limited predictive value. Women who were pregnant again or had carried a subsequent pregnancy to term were the least depressed, suggesting that providers who counsel women should weigh the women's desires to try again along with their physical readiness.

Swanson KM: Predicting depressive symptoms after miscarriage: A path analysis based on the lazarus paradigm. Journal of Women's Health & Gender-Based Medicine, 9(2):191-206. 2000.

Recruitment of Minorities as Research Subjects. African Americans are known to have disproportionately high incidences of disease and illness, yet are under-represented in clinical trials of new methods of diagnosis and treatment. Nurse researchers identified barriers to recruitment and retention of African Americans for scientific study – including historical disregard for African Americans as research subjects. The researchers found that the effective recruitment and retention of African Americans for clinical trials must be based on six fundamental concepts including: historical cognizance; the sanctioning of the investigation by formal and informal community leaders; trust building through openness, thoroughness, and cooperation; mutuality of benefit that assures community confidence in the project; recognition of heterogeneity or diversity within the African American community; and

researcher self-reflection and introspection that questions cultural assumptions the researcher may have. The researchers believe this model will improve the disparity in minority participation in clinical trials.

Dennis BP, Neese JB: Recruitment and retention of african-american elders into community-based research: lessons learned. Archives of Psychiatric Nursing, 14(1):3-11. 2000.

Caring for Patients in a Persistent Vegetative State. Hospital units or freestanding institutions that provide long-term care for persons who are dependent to life support are recent innovations that create a new kind of clinical practice. Using participant observation, this investigator explored end-of-life issues for individuals who are in a persistent vegetative state and are cared for on a specialized, long-term care unit. The author hypothesizes that respirator technologies have created confusion among families and staff about what it means to have a "natural" death, the definition of personhood, what the goals of medical therapy ought to be and what constitutes family responsibility. The author concludes that choices about life and death on these units are affected by the outcomes of resuscitation, respirator technologies, and the clinical care of impaired and deteriorating bodies. Choice replaces nature when the worth, or potential or suffering of the person in a vegetative state is balanced against the comfort and finality of death. This exploration of the care of individuals in a comatose state provides insights about the impacts of new technologies and clinical practices with patients who require life support on long-term care units.

Kaufman SR: In the shadow of "death with dignity": medicine and cultural quandaries of the vegetative state. American Anthropologist, 102(1):69-83. 2000.

Women's Responses to Sexual Violence by Male Intimates. Sexual assault of women by male intimates is an underreported problem with major social, legal and public health implications. Investigators defined a core variable in the framework of women's responses to sexual violence as "forging ahead in a dangerous world." Three aspects of "forging ahead" were identified based on the perceptions of women who have experienced sexual violence from their intimates: telling others, making sense of the violence and creating a safer life. The research provides assessment clues about antecedents of alcohol and drug abuse, as well as a springboard for design of interventions suitable for women victims of sexual violence by male intimates.

Draucker CB, Stern PN: Women's responses to sexual violence by male intimates. Western Journal of Nursing Research, 22(4):385-406. 2000.

The Island of the Color Blind. Pingelap, a coral atoll in the western Pacific that belongs to the Federated States of Micronesia, is the home of a large kindred exhibiting a complete lack of color

vision, or achromatopsia. In this rare, autosomal recessive disorder, patients suffer from photophobia, low visual acuity, nystagmus, and a total inability to distinguish colors. Their retinas contain cone photoreceptors that are viable but fail to generate an electrical response to light. NEI-supported researchers have mapped the gene in Pingelapese islanders to a new gene on chromosome eight. The results establish that classic achromatopsia results from a mutation in this gene and that the gene is not required for vital processes outside the visual system.

Sundin OH, Yang J, Li Y, Zhu D, Hurd JN, Mitchell TN, Silva ED, Maumenee IH: Genetic basis of total colourblindness among the pingelapese islanders. Nature Genetics, 25(3):289-93. 2000.

Outcome for Hepatitis C Virus-Positive Persons is Better Than Expected but Differences in Viral Clearance Exist Between Caucasians and African Americans. In a study that examined clinical outcomes (viral clearance, persistence without complications, or end-stage liver disease) of hepatitis C virus (HCV) infection acquired through injection drug use, researchers followed a cohort of 1667 HCV positive patients with a history of injection drug use prospectively for a median of 8.8 years. Their results indicate that the majority of adults with persistent viremia did not develop clinically demonstrable liver disease. However, viral clearance occurred more frequently among non-African Americans than African Americans.

Thomas DL, Astemborski J, Rai RM, et al: The natural history of hepatitis C virus infection: host, viral, and environmental factors. The Journal of the American Medical Association, 284(4):450-6. 2000.

Increasing Treatment Entry and Retention for Street-recruited Opioid Injectors: The Cost Factor. If not in treatment, long-term opioid drug injectors are at high risk of acquiring and transmitting HIV and other blood-borne infections. Methadone maintenance treatment has been shown to reduce heroin and other drug use and risks for HIV and other blood-borne infections, yet only one of six opioid drug injectors is estimated to be in methadone maintenance treatment at any one time. Therefore, increasing the number of drug treatment admissions is an important way to control the spread of HIV and other diseases. In a study of street-recruited, long-term opioid drug injectors researchers found that addicts offered free methadone treatment were significantly more likely to enter and remain in treatment (nearly twice as long) compared to those who had to pay for it. Free treatment had an even greater impact on drug injectors who had never been in treatment: nearly half of those who had never been in treatment entered when it was free compared to only 23 percent when it was not free. It is commonly believed that paying for treatment provides strong motivation for completing treatment. However, these results show that offering free methadone maintenance treatment to long-term opioid injectors leads to greater treatment entry and retention, especially among injectors who have never tried treatment or who are reluctant to enter treatment.

Kwiatkowski CF, Booth RE, Lloyd LA: The effects of offering free treatment to street-recruited opioid injectors. Addiction, 95(5):697-704. 2000.

Attention Deficit Hyperactivity Disorder and Substance Abuse. There is continued concern but very little data regarding whether or not treating children with ADHD with stimulants, such as Ritalin, can lead to later drug abuse. A new study addressing this issue showed that boys with ADHD who received treatment were significantly less likely to abuse drugs and alcohol when older. The research showed that 75% of the non-medicated ADHD boys had at least one substance use disorder compared to 25% of the medicated ADHD boys and 18% of the boys without ADHD. These results indicate that accurately diagnosing and properly treating ADHD may help prevent emergence of substance abuse in these at-risk individuals.

Biederman J, Wilens T, Mick E, Spencer T, Faraone SV: Pharmacotherapy of attention-deficit/hyperactivity disorder reduces risk for substance use disorder. Pediatrics, 104(2):e20. 1999.

New Reimbursement Classification System for Rehabilitation Care Recognizes Quality While Monitoring Costs. Current medical reimbursement practices have failed to curb rising health care costs and disparities in payment, particularly as they relate to providing care to people with disabilities. In a first-of-its-kind comprehensive effort for rehabilitation services, academic researchers and industry representatives developed a classification system and index that can be applied when paying for services rendered to patients with disabilities or requiring rehabilitative care. This classification system and index considers the difficulty of the cases being treated, and weighs diagnostic information by the extent to which it is expected to affect patients' functional status and their inpatient rehabilitation length of stay. The resulting information should provide clinicians with consistent feedback, allowing them to contain costs while assessing and improving the quality of care received by persons with disabilities.

Stineman MG, Ross RN, Williams SV, Goin JE, Grange CV: A functional diagnostic complexity index for rehabilitation medicine: measuring the influence of many diagnoses on functional independence and resource use. Archives of Physical Medical Rehabilitation, 81(5):549-57. 2000.

Health Care is Not Related to Health Outcomes for Patients with Type 2 Diabetes. To investigate the current relationship between medical care and health status and outcomes, a scientist analyzed a questionnaire and clinical and laboratory data for a representative sample of patients with type 2 diabetes using a cohort from the Third National Health and Nutrition Examination Survey (NHANES III). The survey included measures of health care received (frequency of physician visits, health insurance coverage, screening for diabetes complications, and treatment for high blood sugar, high blood pressure, and abnormal lipid levels in the blood) and measures of health outcomes (high blood sugar, presence of protein in the urine, high blood pressure, and abnormal blood lipid levels). In

all measures of health outcomes, results were not encouraging. The patients had high rates of hyperglycemia, proteinuria, and uncontrolled hypertension and hypercholesterolemia. While health care access and utilization were at high levels, along with screening and treatment for diabetes complications, the failure of these efforts to actually improve health status indicated that other variables might be responsible. The author suggests these variables may include the resistance of diabetes to current therapies, patient self-care practices, physician medical care practices, and characteristics of health care systems in the U.S.

Harris MI: Health care and health status and outcomes for patients with type 2 diabetes. Diabetes Care, 23(6): 754-8. 2000.

Prostate Cancer Outcomes Study. Despite the widespread incidence of prostate cancer – over 180,000 men will be diagnosed in 2000 – controversy still exists about the optimal treatment of all stages of the disease. The ongoing Prostate Cancer Outcomes Study (PCOS), launched in 1994 to investigate variations in the initial treatment of prostate cancer and to describe health-related quality of life outcomes among prostate cancer patients, continues to produce important information about outcomes of the various treatments of prostate cancer. Among its accomplishments to date, the PCOS has demonstrated that specific treatments (radiation therapy, radical prostatectomy, or hormonal therapies) can have detrimental effects on urinary, bowel, and sexual functions, and identified the best predictors of spread of the disease outside the prostate. By collecting comprehensive data on the health outcomes of various treatments for prostate cancer, the PCOS will help men, their families, and physicians make decisions about treatment options.

Potosky AL, Harlan LC, Stanford JL, et al: Prostate cancer practice patterns and quality of life: the prostate cancer outcomes study. Journal of the National Cancer Institute, 91(20):1719-24. 1999.

Gilliland FD, Hoffman RM, Hamilton A, et al: Predicting extracapsular extension of prostate cancer in men treated with radical prostatectomy: results from the population based prostate cancer outcomes study. The Journal of Urology, 163(5):1341-5. 1999.

Stanford JL, Feng Z, Hamilton AS, et al: Urinary and sexual function after radical prostatectomy for clinically localized prostate cancer: the prostate cancer outcomes study. The Journal of the American Medical Association, 283(3):354-60. 2000.

Albertsen PC, Hanley JA, Harlan LC, et al: The positive yield of imaging studies in the evaluation of men with newly diagnosed prostate cancer: a population-based analysis. The Journal of Urology, 163(4):1138-43. 2000.

Potosky AL, Legler J, Albertsen PC, et al: Health outcomes afterl prostatectomy or radiotherapy prostate cancer: results from the prostate canceroutcomes study. Journal of the National Cancer Institute. 92(19):1582-92. 2000.

Estrogen Replacement Therapy and Breast Cancer Risk. Researchers explored the sometimes controversial question of whether menopausal estrogen replacement therapy (ERT) increases a woman's chance of developing breast cancer. In one study of ERT use within the last four years, researchers found that combined estrogen-progestin replacement therapy was associated with a greater risk of breast cancer than estrogen replacement alone, and that both groups had a higher risk than non-users; however, in this study, short-term use (two to three years) was not associated with increased risk, suggesting that women who take menopausal hormones for two to three years are not at increased risk of breast cancer. In a related study, researchers found no increased risk of breast cancer among ERT users with a history of benign breast disease. In light of the favorable effects of ERT on the heart, bones, and overall quality of life, these findings support the use of ERT as a reasonable treatment for menopausal women, at least on a short-term basis.

Schairer C, Lubin J, Troisi R, et al: Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. The Journal of the American Medical Association, 283(4):485-91. 2000.

Dupont WD, Page DL, Parl FF, et al: Estrogen replacement therapy in women with a history of proliferative breast disease. Cancer, 85(6):1277-83. 2000.

Risk of Bipolar Relapse with Lithium Discontinuation Increased in Post-Partum Period, But Not Pregnancy. Comparing relapse rates while off medication (lithium) for pregnant and nonpregnant women with bipolar disorder during 40 weeks of pregnancy and 24 post-partum weeks (and during an equivalent period of 64 weeks for nonpregnant subjects) researchers found no differences in relapse rates between women for the initial 40 weeks. However, during weeks 41-64 (the post-partum period) the pregnant mothers were three times more likely to have a recurrence of their illness. This finding suggests that the post-partum period is specifically associated with an elevated risk of relapse of bipolar disorder for women who discontinue lithium maintenance therapy, and suggests the need to design and study alternative treatment methods.

Viguera AC, Nonacs R, Cohen LS, Tondo L, Murray A, Baldessarini RJ: Risk of recurrence of bipolar disorder in pregnant and nonpregnant women after discontinuing lithium maintenance. American Journal of Psychiatry, 157(2): 179-84. 2000.

Behavior Modification for Urinary Continence. Direct costs of treatment for urinary incontinence in the United States have been estimated to exceed $16 billion per year. Growing evidence suggests that comparatively inexpensive and highly effective behavioral therapies should be the first line of treatment for most women. After ruling out associated medical conditions that require treatment, clinicians can implement noninvasive strategies to increase bladder capacity and strengthen muscles in the pelvic floor. Numerous recent studies have shown that these strategies, which combine practical information about pelvic anatomy with exercises to develop capacity and control, are more effective in

reducing incontinence than pharmacologic and surgical interventions. Health-care providers can readily incorporate these strategies into everyday practice with women.

Sampselle CM: Behavioral intervention for urinary incontinence in women: evidence for practice. Journal of Midwifery & Women's Health, 45(2):94-103. 2000.

STORIES OF DISCOVERY

Methamphetamine Abuse: Confronting a Public Health Crisis

Methamphetamine – what was in the 1930s a promising new treatment for nasal congestion, is now in the 1990s, a growing public health menace. We now know that methamphetamine is not only a powerfully addicting stimulant, but a drug that it is highly toxic to human brain cells when abused. These properties, coupled with the fact that it is easy to make with commonly available ingredients make it a looming public health problem for our country.

Methamphetamine was first developed in 1919 as an amphetamine derivative by a pharmacologist in Japan. However, it was not until the 1930s that the pharmacological properties of the amphetamine family of compounds were discovered. Initially, amphetamines were found to have the potent ability to constrict blood vessels. In fact, amphetamine was initially marketed in 1932 as Benzedrine, an inhaler designed for the relief of nasal congestion. Users of these over-the-counter inhalers soon discovered that the amphetamine contents of these inhalers were powerful central nervous system stimulants because, as scientists later discovered, of their ability to activate the dopamine system in the brain. Soon abusers were disassembling the inhalers to retrieve the amphetamine containing paper inside, since these drugs activate the reward or pleasure circuit.

There have been several waves of amphetamine and methamphetamine abuse in this country since then. During the 1950s and 1960s, methamphetamine and amphetamines were being diverted from pharmaceutical products and being abused by truck drivers, students, and housewives as insomniac and weight loss agents. Intravenous use of methamphetamine became popular among white, young adults, particularly in the Haight-Ashbury district of San Francisco in the late 1960s and early 1970s. As a result of the increasing abuse, the Controlled Substance Act was passed in 1970 and dramatically changed the availability of pharmaceutical amphetamines. As pharmaceutical supplies of amphetamines became more difficult to obtain, the purity of street methamphetamine declined dramatically, with caffeine and ephedrine often substituted or sold as methamphetamine. Because the production of methamphetamine does not require special knowledge or expertise in chemistry, there began to be a reemergence of the illicit use of methamphetamine in the 1980s.

The use of methamphetamine was confined in the 1980s primarily the West Coast. A number of indicators, including those from the NIH's Community Epidemiology Work Group (CEWG) and a new Multi-site Assessment of Methamphetamine Use, are clearly showing that the abuse of this drug is now spreading throughout the Midwest and into other areas of the country and emerging in cities and rural settings thought previously to be untouched. Not only is it spreading to new areas, but its use is increasing among populations not previously known to use this drug. Recipes for methamphetamine have been available and circulated among numerous sources. In the 1990s, the Internet has increased

access to recipes and instructions for producing methamphetamine, and has also contributed to its spread.

The increasing use of this drug is of particular concern because of recent research from a number of sources demonstrating the neurotoxic effects of the drug. In addition, because of sloppy production, what is often sold as methamphetamine can contain a number of toxic contaminants including lead, chloroform, and sulfuric acid. In the 1980's evidence was accumulating that methamphetamine was toxic to dopamine and serotonin containing neurons in animals given relatively high doses. In 1997, it was shown that non-human primates exposed to methamphetamine doses routinely used by human abusers, resulted in profound damage to both the brain's dopamine and serotonin neurotransmitter systems. These effects also appear to be long-lasting in the rhesus monkey. for example, there are data showing that changes persist for over 3 years. There is now alarming evidence of toxicity in humans. In 1998 and 1999, using state-of-the-art neuroimaging technology, investigators found that methamphetamine abusers who have been abstinent from methamphetamine for several years show significant decreases in the numbers of brain dopamine transporters, similar to the data that were first seen in non-human primates. These long-lasting neurochemical effects may be partly responsible for the severe behavioral abnormalities that accompany prolonged abuse of this drug.

In addition to characterizing the toxicity, researchers have also been striving to understand the causes of toxicity. Research in cell culture and animal models has proposed several mechanisms of cellular toxicity and has identified many compounds that seem to protect animals against methamphetamine toxicity. NIH is pursuing the development of these compounds into medications that may be effective in treating methamphetamine addiction. Understanding how methamphetamine's biochemical and pharmacological actions can lead to cell death is critical to restoring cognitive function to methamphetamine-impaired individuals and for developing medications for reversing or treating addiction. This new knowledge may also have far-reaching effects in the treatment of stroke, Parkinson's disease and other neurodegenerative diseases in which the common neurotoxicity mechanism is operative.

Looking in Cells for the Sources of Alcoholism

Alcohol researchers are finding that at least part of the source of alcoholism can be found in certain cellular activities. Cells produce substances the body needs to function, and hundreds of synchronized biochemical reactions – "pathways" – take place in them. Ultimately, these pathways influence people's physical appearance and behaviors, including, scientists believe, drinking behaviors. Identifying pathways involved in drinking behaviors will help researchers design medications that biologically interrupt them.

None of these cellular activities happen in a vacuum. Constantly bombarding cells are molecular stimuli that "tell" cells what's needed in the rest of the body. For example, some stimuli trigger conditions for nerve cells to carry electrical impulses to one another; others act as chemical messengers and receivers between nerve cells, activating certain pathways in them. Among these pathways is one, the cyclic AMP or "cAMP" signaling system, that occurs inside cells and is crucial in signaling the initiation of yet more cellular activities.

Alcohol researchers have built on a body of work on cAMP, conducted by investigators from a variety of fields, that began in the 1950s. These earlier investigators identified much of how the cAMP signaling system works under normal conditions. They found that the cAMP pathway acts on protein kinase A (PKA), a protein that alters the functions of other proteins by adding a phosphate molecule to them, with important results. Proteins are the chief regulators of cellular activities, and many become active only on addition of this phosphate molecule. The result is that cells can control the timing and placement of proteins' activities with the necessary precision. These activities are possible because cAMP causes the subunits that comprise PKA to split, activating one of them, the catalytic subunit. On activation, this subunit goes throughout the cell's interior (cytoplasm), adding phosphate molecules to proteins at the right time and place.

Equally important is the catalytic subunit's subsequent migration into the nucleus of the cell. This is where genes, the DNA blueprints that tell cells what kinds of proteins to make, reside. Here, the subunit adds phosphate to a molecule that turns on a wide variety of genes, causing them to go into action, which then causes the cell to produce the proteins that each of the genes encodes. The catalytic subunit leaves the nucleus and returns to the cytoplasm, where it re-attaches to the other subunit (PKA-R) from which cAMP had separated it earlier, making it inactive again. When the cell receives the right external stimulus, cAMP causes the PKA subunits to split up again, and the cycle repeats.

For the most part, external stimuli tell cells what they need to do by temporarily binding onto docking sites made of protein molecules that are on, or embedded in, the protective membranes that surround cells. This binding activates a cell-membrane protein that makes cAMP.

To alcohol researchers, nowhere are these activities more important than in the nervous system – the brain – because this is where alcohol exerts its effects on behavior. In the 1970s, alcohol researchers found that rodents' brain tissue and cultured nerve cells increased in cAMP levels when they were exposed to alcohol. The implications were significant: cAMP performs crucial functions not only in the cytoplasm of the cell, but also in its nucleus, where genes reside. These early researchers found that *acute* alcohol exposure (a single bout of heavy drinking, for example) raised cAMP levels by affecting the activity of the cell-membrane protein that makes it. Subsequent studies revealed that cells adapted to *chronic* alcohol exposure by reducing cAMP levels, resulting in a decrease in events farther along in its biochemical pathway. Chronic alcohol exposure reduced the amount or activity of the proteins that produce cAMP.

Within the past 5 years, researchers have demonstrated that alcohol promotes the rapid migration of PKA's catalytic subunit – the one involved in turning genes on, thereby activating protein production by cells – to the nucleus of the cell. Normally, the catalytic subunit leaves the nucleus quickly when its function there is done, but researchers found that it stayed in the nucleus as long as alcohol was present. Thus engaged, the catalytic subunit is unavailable to return to the cell's cytoplasm, to add phosphates to other proteins; these proteins are deprived of the normal alterations that would enable them to work properly. The catalytic subunit's prolonged presence in the nucleus also results in an excess of its normal activity there; that is, to add phosphate to a substance that turns on genes. Scientists are not sure of the results of this excessive turning on of genes, but they believe it may be responsible for neurophysiologic changes that lead to alcohol dependence. Research continues in this area.

At the same time scientists were exploring the involvement of cAMP signaling in alcohol dependence, others were exploring the role of this system in alcohol's acute intoxicating effects. They took a different tack. Epidemiology studies had revealed that half of the risk for alcoholism is genetic. Fruit flies are valuable research tools, because their genetic makeup is similar to that of humans, and genetic findings in one species provide clues about where to look in the other. These scientists knew that the fruit fly's cAMP signaling system also is similar to humans'. They demonstrated that mutations in several genes involved in the cAMP system increased fruit flies' sensitivity to alcohol. Proper functioning of the cAMP signaling system, as demonstrated by scientists, is required for control of sensitivity to alcohol in the fly. Previous studies had revealed that a person's baseline sensitivity is directly related to his or her risk for alcoholism.

The most recent study of the cAMP pathway's role in alcohol dependence demonstrated that mice engineered to have a gene mutation that inactivates PKA-R – the PKA subunit that temporarily disables the catalytic subunit – are resistant to alcohol's effects and drink more of it than do normal mice. Thus, cAMP signaling is involved in control of alcohol sensitivity in both mice and flies, an indication that it is likely to play the same role in humans.

Alcohol is unique in the pervasiveness and complexity of its actions in the nervous system, and other pathways are likely to emerge in the search for the origins of drinking behaviors. Taken alone, none of the studies described here constitutes the last word in what causes people to become alcoholic. Taken together, however, they lend considerable weight to the assertion that the cAMP pathway is a front-runner in the search for mechanisms that lead to alcoholism.

This is how stories of discovery grow in scientific research: in steps that build on steps that came before. Ultimately, they provide scientists with the information they need to design interventions for diseases like alcoholism, an illness that destroys minds, bodies, and families.

Identification and Characterization of a Family of Bitter Taste Receptors

On its own, the sense of taste provides animals with information important for survival, from the deliciously sweet-taste of a perfectly ripe peach to the intense bitterness so often associated with toxic or spoilt food. The taste for sweetness and salt and the rejection of bitter that remain from our evolutionary past also present major problems. Thus despite its role (in combination with other senses, most notably smell), in providing the daily pleasure of eating, aspects of human taste also have growing implications in public health. Of all the senses, taste remains the most mysterious, for example we still do not know how different tastes are detected and discriminated in the oral cavity. However, the mass of genetic information flooding in with the human genome project has now helped scientists to start closing this gap in our knowledge.

In the past, progress in understanding taste has been hampered by the very small number of receptor cells. These cells, grouped in taste buds scattered on the surface of the tongue and other regions of the oral cavity, are where taste detection and discrimination takes place. It is believed that taste responses begin when sapid compounds (or tastants) bind to specific proteins, the taste receptors, on the surface of these cells. This binding triggers the cells to activate nerves and transmit the information to the brain. In other words, the brain receives information about the pattern of taste cells that are stimulated and our ability to discriminate between two compounds requires that they stimulate different subsets of taste receptor cells. Thus scientists expect taste receptors to be found on the surface of subsets of taste receptor cells. Last year the first two proteins with these properties were discovered using modern molecular techniques, but it is not yet clear what role they play in taste. Now, using the data generated from the human genome project a whole family of new receptors has been discovered.

For the last 30 years, it has been appreciated that the ability of mice and humans to detect certain bitter compounds is genetically determined. The approximate positions of several of the genes controlling bitter taste perception have been mapped in mice and, last year, the position of one was also mapped in humans. Therefore scientists searched the growing wealth of DNA sequence information, looking for possible receptor genes in this region of the genome. This search revealed just such a sequence in the right part of the genome and led to the discovery of a whole family of related genes distributed at other sites in the human genome. Intriguingly, when the corresponding receptor genes from mice were mapped, many corresponded with bitter taste loci. Additional circumstantial evidence suggested that these might be taste receptors, for example as expected they are selectively expressed in the membranes of subsets of taste receptor cells. But what really sets this discovery apart from the earlier investigation is the proof that at least some members of the new gene family actually function as taste receptors – that is, they trigger cellular activity after binding to bitter compounds.

In biology, bitter taste appears to be an almost uniform warning of toxicity. Indeed many different naturally occurring poisonous compounds taste bitter to humans and are aversive stimuli for laboratory animals. However, structurally these molecules have little or no similarity and so it has never been clear

why they should all taste bitter. What do these new receptors tell us about how bitter taste works? It turns out that each receptor cell that contains one receptor actually carries the entire family of these receptors on its surface. Therefore, the pattern of cells stimulated by a bitter tasting compound that binds to one of these receptors is indistinguishable from the pattern produced by a different bitter tasting compound binding to a different receptor. Since the brain receives and interprets information about the patterns of cells that are activated, this provides a logical explanation for the uniform bitter taste of toxins.

These findings may lead to more fundamental advances in our knowledge about the organization of the nervous system and its responses to the environment. Identification of functionally defined taste receptors provides molecular tools to mark specific taste receptor cells, define signaling pathways, dissect receptor specificity, generate topographic maps, and trace the neuronal connectivity circuits. A deeper understanding of taste mechanisms could also translate into improved therapeutic regimes. For example, researchers have found that certain drugs for AIDS, heart disease, and depression taste so bad or so ruin the flavors of food that patients abandon life-saving medications. The ability to block the bitter taste receptor could dramatically improve compliance with life-saving drugs. Moreover researchers have also shown that the sense of taste is linked to a predisposition toward dietary choices that may contribute to obesity and health related problems including increased risk of diabetes, heart disease and stroke. Variations between bitter taste receptors within the human population may provide a valuable tool for predicting the likely dietary choice of individuals, and their likelihood for developing certain diseases.

Simian Immunodeficiency Virus Models the Human AIDS Virus

More than 20 years ago – as concerns about a mysterious and deadly new disease known as acquired immunodeficiency syndrome (AIDS) began to sweep the Nation – scientists at the California Regional Primate Research Center (RPRC) were puzzling over a new outbreak of infections that were decimating their monkey colonies. Inexplicably, dozens of animals became dangerously thin and weak, and many developed malignant tumors, severe herpesvirus or bacterial infections, anemia, or inflammation of brain tissues. Most affected animals were dead in a matter of months. Meanwhile, on the other side of the country, researchers at the New England RPRC near Boston noticed a similarly disturbing trend among their macaque monkeys. As investigators launched a search for the disease-causing agent, they little suspected the enormous impact their efforts would later have on understanding AIDS virus infections in humans and developing methods for its treatment, control, and prevention.

The more researchers learned about the monkey syndrome, the more obvious it became that the human and simian disorders were strikingly similar. Both diseases were marked by a weakened immune system that laid the body vulnerable to a variety of infections that normally did not cause disease. Scientists on both coasts began to suspect that studies of this monkey immunodeficiency disorder, or simian AIDS, could provide otherwise-unobtainable insights into the progression of human AIDS.

Back in 1980 no one knew what caused AIDS – suspects ranged from a variety of viruses to a recreational drug known as poppers. Without knowing the causative agent, it was impossible to study or diagnose the earliest stages of human infection. A critical lead came in 1983, when two teams of scientists independently isolated a new human retrovirus – with a genome consisting of RNA rather than DNA--from the tissues of AIDS patients. Skeptics questioned whether this virus, now known as the human immunodeficiency virus (HIV), could produce the severe immunodeficiency characteristic of AIDS. Only two other retroviruses were known to infect humans, and both of these caused cancer. Crucial support for a retroviral basis of AIDS in primates came when RPRC investigators isolated and identified a new virus, which they dubbed simian retrovirus-1 (SRV-1), from the tissues of AIDS-affected animals in 1984. When the isolated virus was injected into healthy monkeys, the animals developed an AIDS-like disorder within 2 to 4 weeks. Studies of simian AIDS offered the first opportunity to track the immune system's initial response to this highly contagious retrovirus.

Although SRV-1 triggers an AIDS-like disease, researchers were disappointed to learn that the simian virus was unexpectedly different – both structurally and genetically – from HIV. Scientists at the New England RPRC decided to systematically search for HIV-like viruses (lentiviruses) in their primate colonies. They eventually isolated the virus now known as the simian immunodeficiency virus (SIV) from several species. As the closest known relative of HIV, SIV not only looks similar to the human virus under the microscope, but also has similar genes, biological properties, and effects on the immune system. Like its human counterpart, the simian virus particularly infects and destroys white blood cells known as T-helper (or CD4) cells. Because these cells are required for the body to mount an effective

immune response against disease-causing agents, their destruction explains the profound immunodeficiency seen in humans and monkeys with AIDS.

SIV infection of macaques is now widely considered the best animal model for human AIDS and is used by hundreds of AIDS researchers worldwide. In many cases, SIV infection progresses to AIDS rapidly – in a matter of months rather than the decade typically seen in HIV-infected humans – which makes the animal model suitable for timely investigation of the disease process. The model has proven especially useful for evaluating potential AIDS vaccines. Monkey studies allow scientists to challenge vaccinated animals with deadly strains of virus to determine if the vaccine is protective. Comparable experiments could not ethically be performed on humans.
Some SIV vaccines completely protect animals from even the most deadly variants of SIV. These vaccines are made of live, but weakened (or attenuated) strains of SIV in which one or more viral genes are deleted. Vaccinated animals have remained virus-free and healthy for years after complete viral challenge. Live attenuated AIDS vaccines may be deemed too risky for human use, since the weakened virus may still be capable of causing disease in some recipients. However, the monkey studies offer proof of principle that it is possible to develop an AIDS vaccine that can prevent infection. Researchers are now scrutinizing the immune responses of vaccinated monkeys to identify the factors that keep SIV infection at bay.

Many experiments suggest that antibodies alone are incapable of thwarting an SIV attack, and that protection against the AIDS virus will also depend on activation of white blood cells known as killer (or cytotoxic) T-cells. These cells latch onto and destroy virus-infected CD4 cells. NIH-supported investigations at RPRCs show that killer T-cells come on so strong in the first weeks of infection that they nearly eliminate SIV by homing in on the viral protein Tat, which is displayed on the surface of infected CD4 cells. A few viruses, however, have mutant versions of Tat and so slip unnoticed past the pre-programmed killer cells. Eventually, these mutant viruses are able to repopulate the animal's bloodstream and cause full-blown infection. Identification of specific Tat mutations may assist the design of effective AIDS vaccines. Scientists have also identified the portions of the viral proteins Nef, Gag, Tat, and Env that are displayed on infected cells. Monkeys immunized with these peptides, called epitopes, generate strong cellular immune responses, suggesting that these peptides could be used in vaccines against AIDS.

Studies of SIV in macaques have also shed light on the factors that affect transmission of the AIDS virus from one individual to another. In humans, HIV is most often transmitted when mucosal surfaces are exposed to infected fluids, usually during sex or birth. Monkey studies allowed scientists to identify the mucosal cells in females that are initially infected during heterosexual transmission of the virus. The SIV model also confirmed that the virus could be transmitted to newborns who swallow amniotic fluids or breast milk from infected mothers. These discoveries open new opportunities for blocking HIV transmission with drugs, vaccines, or other precautions.

The SIV model also enhances understanding of the brain and nerve damage that often accompanies AIDS. Although HIV-infected patients are now living longer thanks to improved therapies, some clinicians fear that these longer lives may be marred by AIDS-associated dementia. SIV experiments suggest that infection of blood cells known as monocytes and macrophages play a critical role in transporting the virus across the blood-brain barrier, and recent investigations of HIV infection in humans support these conclusions. Therapies that target these cells may therefore help limit the risk of developing AIDS dementia.

The sudden appearance and rapid spread of human AIDS illustrate how quickly a virus can infect a new species and cause a global pandemic. Ongoing studies are piecing together the factors that enabled SIV in nonhuman primates to jump to human hosts and cause such a devastating disease. One puzzling paradox is that many species of monkeys and apes are infected in the wild with their own distinct variants of SIV but show no disease symptoms and apparently do not mount immune responses against the viruses. This unique tolerance is not yet fully understood but might help scientists understand the mechanisms of HIV pathogenicity – and how to protect against it. Such investigations may also offer clues to preventing or reducing cross-species transmission of other emerging viruses, such as the West Nile virus, which naturally infects birds but has recently killed several people in the United States.

The knowledge gained from studies of SIV demonstrates the importance of studying diseases that arise spontaneously in animals. Because scientists were alert to changes in the health of their nonhuman primate colonies, and because they had access to unique scientific resources and expertise at the RPRCs, they were able to develop an animal model that continues to provide critical insights into the understanding and treatment of human AIDS.

The Speed of Sound:
Motor Protein of Cochlear Outer Hair Cells Identified

Millions of Americans, especially middle-aged and older individuals, suffer from mild to moderate hearing loss. It is likely that a defect in or destruction of the most sensitive elements in the inner ear, the hair cells, causes this type of hearing deficit. Inner ear hair cells are sensory receptor cells that give humans and other mammals the remarkable ability to hear. Sound travels to the ears, down the ear canal, through the bones in the middle ear and into the inner ear, where the outer hair cells amplify the mechanical vibrations produced by the sound. The amplification of sound by the outer hair cells occurs through a process known as electromotility. When the cell responds to sound, molecules pass through the cell's membrane causing electrical changes in the cell allowing it to rapidly change its length and stiffness. In response to the sound-evoked electrical changes, the slender cylindrical outer hair cell will alter its length by as much as five percent. The length changes amplify the vibrations, which are sensed by the inner hair cells that send auditory information to the brain. When outer hair cells are lost or destroyed, hearing becomes insensitive, closely spaced frequencies can no longer be discriminated, and the immense range of sound intensities can no longer be processed.

Scientists have hypothesized that a highly specialized motor protein in the cell's membrane drives outer hair cell motility. As soon as the outer hair cell electromotility process was discovered approximately 15 years ago, scientists began a search to identify the motor molecules that are involved. The difficulty, as with most research involving the ear, is working with the delicate organs of the inner ear, encased in some of the hardest bones in the body, and the small number of sensory hair cells. With support from the NIH, scientists have recently isolated the protein responsible for outer hair cell electromotility. In order to search for the gene that codes for the motor protein, the scientists arduously isolated approximately 1,000 outer hair cells and a similar number of inner hair cells from gerbils. The scientists determined that these sensory cells would express mostly the same genes, but that certain genes will be expressed in either the outer or inner hair cells. Since only outer hair cells are involved in electromotility, the genes that code for the motor protein should be expressed in outer hair cells alone. Several unique clones were identified and one of these, *Prestin*, later proved to be the gene coding for the motor protein. The name *Prestin* (from the musical term presto, indicating a rapid tempo) was selected to emphasize one of the most interesting features of this protein in the cellular motor process, its speed in changing the length of outer hair cells. Outer hair cells can elongate and contract at rates close to 100,000 times a second!

To prove that *Prestin* is the correct gene, scientists expressed prestin in cells that do not normally manufacture the protein and showed that prestin-producing cells exhibited electromotility. This result functionally identified prestin as the long sought motor protein.

With the gene that is key to outer hair cell function determined, scientists are asking many new questions about how the *Prestin* gene impacts hearing. Are there naturally occurring mutations of *Prestin* that are

responsible for certain types of hearing impairment in humans? If so, will it be possible to use gene therapy to restore *Prestin* function in hearing-impaired individuals who have a defect in the gene? What are the consequences in the auditory system of mice that do not express the prestin protein? Do mice that express too much of it exhibit hearing abnormalities? Future research on *Prestin* should lead to significant advances in the understanding of the auditory system, and may lead to the development of new therapeutic measures for hearing impairment.

The Declining Disability of Older Americans

As recently as 20 years ago, scientists predicted technology would save people from dying without curing them, producing a pandemic of old age disability and an exponentially increasing burden of health care services and costs. In the past few years, however, it has become clear that such conventional wisdom is wrong. People are living longer and healthier lives, and the potential for working longer and needing less medical care in the last years of life is very real. These trends are occurring in both the U.S. and in Europe, and are therefore global in nature.

The ability to make reasonably accurate projections with regard to both life expectancy and disability has obvious implications for maintaining the solvency of such large national programs as Social Security and the Medicare Trust Fund. In the mid-1970s, the Social Security Administration made life expectancy projections based on the assumption that mortality would be static, and consequently life expectancy would not improve over time. It soon became clear that such an approach was not accurate. In 1982, the National Commission on Social Security Reform was established to determine how to modify Social Security funding so it would remain solvent in the face of projected life expectancy increases at ages 65 and over. The commission mandated an increase in the normal retirement age from 65 to 67 years in increments from 2000 to 2022. This was done without clear evidence that life expectancy increases at ages 65 and over were associated with significant improvements in health at later ages.

In 1993, a National Academy of Sciences panel found suggestive evidence from the 1982 to 1989 National Long Term Care Study (NLTCS) of chronic disability declines. Confirmation required a longer time series. In 1994, new NLTCS data confirmed that a decline was occurring in chronic disability. The chronic disability rates declined 1.1% per year from 1982 to 1989 and 1.5% per year from 1989 to 1994 – suggesting an accelerating trend. It was estimated that at least 1.2 million fewer older Americans were disabled in 1994 than there would have been if disability rates had not improved since 1982. More recent preliminary findings from the NLTCS suggest a continuation of the decline and perhaps even a reduction in the absolute number of elderly disabled persons.

These findings were of great potential significance in terms of identifying and addressing causes of disability, as well as informing national health care policy. It is therefore important that confirmation of the trend toward decreased disability has subsequently come from independent studies and investigators. For example, an analysis of data from the Survey of Income and Program Participation (SIPP) in 1998 showed a reduction in disability rates from 1984 to 1993 in every age group of elderly Americans 50 years and over. The SIPP per year declines, at 0.9 to 2.3%, were even larger than in the NLTCS, were relatively more rapid above age 80, and also occurred for seriously disabled persons. Analysis of the 1991 to 1996 Medicare Current Beneficiary Survey also showed disability declines more rapid than in the 1982 to 1994 NLTCS. Declines of 0.9% per annum were found in the 1983-

1994 National Health Interview Survey. All of these corroborating findings have helped establish that the disability decline may reflect real improvements in underlying physiological health.

The long-term implications of disability decline depend in large part on whether the trend continues and at what pace. On the basis of current disability rates, demographers estimate far fewer working age people in the future per disabled person, but if disability rates continue to decline by 1.5% per year, there would be more working age people in the future per disabled older person. It has been projected that between now and the year 2030 expected increases in education attainment among the elderly will continue to contribute to improvements in functioning, although at an increasingly slower rate since education levels among the elderly will not be rising so rapidly as previously. Related trends that are likely to stimulate continued disability decline include improvements in health related behaviors, the availability and effectiveness of assistive devices, and the treatment of conditions that lead to disability. At this time, there is no consensus about the likely pace of future disability decline.

Research has begun to focus on plausible models to explain the recent declines in disability in order to identify specific interventions, behavioral changes, and survival attributes that can accelerate the trend toward decreased cumulative disability, postponed onset of disability, regaining of function, and improved quality of life. Since the molecular and genetic bases of many chronic diseases are only beginning to be identified, systematic reductions in chronic degenerative diseases have yet to occurred. The pace of research and clinical activity is, however, increasing rapidly. In 1997, 700 drugs were in clinical trials in the U.S. In 1998, 1,200 drugs were in clinical trials with 305 in critical phases of investigation.

A broad research effort is also under way on the long-term economic consequences of the disability decline and the adoption of new medical technologies. Since people are physically capable of working longer,many may defer retirement and continue working until older ages. This has implications for Social Security, the Medicare Trust Fund, and for the overall productive capacity of the economy. It has been argued that if a 1.5% annual decline in chronic disability were maintained for the next 40 years, the Medicare Trust Fund would remain solvent. Some also contend that biotechnological innovation has and will continue to stimulate health improvements that may ultimately lower health care costs. Declining rates of chronic disability may also moderate the burden of caregiving, including the informal care provided within families, the care provided through home health services, and the care provided in long-term care institutions.

The far-reaching potential implications of disability decline have stimulated a broad research agenda. Clearly, research on disability has helped change the way aging is viewed. What was once seen as a one-dimensional process of biological senescence is now seen as a complex multi-dimensional process where components change at different rates, and are amenable to multifaceted interventions. New paradigms in biotechnology offer promise for future acceleration of these trends in the decline of disability.

Learning From Songbirds About Adult Brain Cell Generation

As recently as two years ago, most neuroscientists believed that all the brain cells that a person would have throughout life were created during a narrow window of time, before birth and during the first years of life. The conventional wisdom held that no new nerve new, or neurons, could be generated after early childhood. People thought that the brain's neurons – unlike cells of other bodily organ systems, such as skin, which continuously generates cells to replace those that die or are injured – were irreplaceable. Only recently has research shown that the brain indeed can add nerve cells during adult life through a process called neurogenesis. The implications of these findings for therapeutic interventions are potentially staggering and, currently, an intensive and prolific area of investigation.

The first solid evidence that adult brains may be able to add nerve cells emerged in the 1980s from basic animal research involving songbirds. Researchers showed that during mating season, the number of neurons in certain areas increase and decrease in cadence with a bird's seasonal need to recall his mating song. Previous research had suggested that a low level of neurogenesis occurs in certain regions of the rodent brain, including the hippocampus (a brain region required for the formation of conscious memories and learning) during the adolescent period, long after the generation of neurons in most brain areas had ceased. But the unequivocal evidence of robust growth in songbirds reinvigorated the search for neurogenesis in higher animal models. Investigators went on to show that not only does the rodent brain in fact continue to generate neurons during late adolescence, but also that this process continues even into adulthood.

Technical developments in brain imaging spurred the search for the elusive evidence of neurogenesis, and numerous laboratories have begun to develop a clearer, encouraging picture of the phenomenon. In 1998, NIH-supported investigators working with monkeys found hippocampal generation of neurons in adulthood. Within a few months of that report, other researchers demonstrated the birth of new nerve cells in the adult human brain. Today, work in laboratories nationwide is finding that the rate at which new nerve cells are generated – and factors that prevent their development – can be influenced by environmental factors. Studies have shown, for example, that stress inhibits the formation of new neurons.

Findings that neurogenesis occurs across the lifespan are changing profoundly the way neuroscientists think about the nervous system and, more specifically, about possible future interventions for disorders such as depression, schizophrenia, and autism. With respect to the latter two conditions, an open question now is whether there is an actual loss in number of cells or, conversely, whether the number of cells remains constant while the density of certain cellular extensions that play a critical role in processing information diminishes. Recent data obtained from brain imaging studies reveal a relative decrease in hippocampal volume – that is, the number of neurons in that particular brain region – in patients with recurrent depressive illness. The association of the hippocampus with learning and memory poses the intriguing possibility that stress-induced changes in hippocampal neurogenesis may be an important

factor in precipitating episodes of depression. If this proves to be the case, the possibility exists that various factors, including medications and new learning, or cognitive frameworks, could trigger the brain to produce certain neurochemicals that in turn stimulate hippocampal neurogenesis and, ultimately, promote recovery from depression

The adult cells that promote, or permit, neurogenesis are related to – but critically different from – those much-in-the-news fetal stem cells. These cells were long thought to occur only during embryonic development, at which time they divide, differentiate, and give rise to mature tissues and organs. The new findings about neurogenesis point to the existence of adult stem cells and the exciting possibility that they may prove to become our body's universal repair kit. Adult stem cells now have been isolated from several different tissues in the body, including the brain. Reports being published daily indicate these cells to be capable of producing any type of cell in the body. Scientists hypothesize that adult stem cells, unlike their embryonic counterparts, could be derived from any individual. Not only would such sourcing of stem cells circumvent embryonic stem cell ethical controversies, but also would also offer a means of avoiding tissue rejection.

Right now, investigators across the country and throughout the world are working feverishly to learn how to stimulate these cells and to control their differentiation into specific classes of tissues. Their success will buttress hope that many currently incurable disorders could benefit from specific types of stem cell related therapy such as tissue replacement or transplantation.

Sequencing the Human Genome: Our Genetic Instruction Book

The Human Genome Project was started in 1990 and has, from its beginning, enjoyed significant success. Many of the project's initial goals have been achieved, including building maps to localize and order the position of genes in both the human and mouse genomes, and sequencing the genomes of model organisms including the bacterium *E. coli*, baker's yeast, and the roundworm *C. elegans*. Building on those successes, this year HGP scientists reached a historic milestone: the completion of a working draft sequence of the human genome.

The Human Genome Project was initiated for medical reasons. Sequencing the genome is far from an esoteric scientific exercise. Determining the complete genetic blueprint of humans will greatly accelerate the identification of the genes underlying many human diseases, including complex diseases that represent the greatest health burden to the U.S. population. It is an early and vital step in the understanding of virtually all diseases.

Throughout history, medicine has been conducted, for the most part, without knowledge of the fundamental underpinnings of disease. In the case of genetic diseases (and most diseases are influenced by genetic factors) this requires the identification of the gene, or genes, whose sequence is altered in predisposed or affected individuals. Identifying those genes is the first step to a more profound understanding of the biological basis of disease and this, in turn, will lead to much more effective and inexpensive ways to diagnosis, treat, and prevent disease.

On June 26, 2000, leaders of the public Human Genome Project and Celera Genomics Corporation announced that both had successfully completed the initial sequencing of the human genome. This historic scientific milestone was announced with President Clinton at a White House event. It included a satellite link to Prime Minister Tony Blair and genome leaders in the U.K. who contributed significantly to the public effort.

Producing the working draft involved two tasks: placing large fragments of DNA in the proper order to cover all of the human chromosomes, and determining the DNA sequence of these fragments. The assembly reported on June 26th consisted of overlapping fragments covering 97 percent of the human genome, of which sequence has already been assembled for approximately 85 percent of the genome.

The production of human genome sequence skyrocketed over the past year. During this time, the international sequencing consortium produced 1000 bases a second of raw sequence – 7 days a week, 24 hours a day. The average quality of the "working draft" sequence far exceeds the consortium's original expectations for this intermediate product.

The "working draft" is substantially closer to the ultimate "finished" form than the consortium expected at this stage. Approximately 50 percent of the genome sequence is in near-"finished" form or better, and

24 percent of it is in completely "finished" form. The average accuracy of all of the DNA sequence in this assembly is 99.9 percent.

The sequence information from the public project has been continuously, immediately and freely released to the world, with no restrictions on its use or redistribution. The information is scanned daily by scientists in academia and industry, as well as by commercial database companies providing information services to biotechnologists.

The public Human Genome Project and Celera Genomics used different, but complimentary, approaches to sequencing the human genome. The public project used a 'hierarchical shotgun' approach in which individual large DNA fragments of known position are subjected to shotgun sequencing. Celera used a "whole genome shotgun" approach, in which the entire genome is shredded into small fragments that are sequenced and put back together on the basis of sequence overlaps.

Public and private research teams are committed to publishing their genomic data simultaneously and after publication, both teams will join together for an historic sequence analysis conference. Together, they will examine what scientific insights have been gleaned from both efforts, and how we can most judiciously proceed toward the next majestic horizons.

A Century of Fruit Fly Research Sheds Light on Human Health and Disease

In March 2000, scientists announced that they had unscrambled the genetic code of a tiny fruit fly called *Drosophila melanogaster*. In doing so, thousands of previously unknown genes were uncovered. Within a week, dozens of stories about the scientific tour de force appeared in newspapers and magazines around the world. Why did the media pay so much attention? The *Drosophila* genome was the largest gene code unscrambled thus far, and the completion of this project in record time validated the controversial method later used to sequence the human genome.

With important genes, Mother Nature has demonstrated an exquisite sense of economy: 177 of the 289 human genes that when "misspelled" are known to cause diseases in people have direct counterparts in the fruit fly. A complete catalog of fly genes is an extraordinary tool that will help researchers understand how genes work – not only in flies but also in humans and other organisms. The achievement is a boon to the thousands of scientists who study human health using the fruit fly as a model, one of a host of valuable model systems that continue to aid researchers in making breakthroughs in preventing and curing disease.

Drosophila became a model system for studying how genes work nearly 100 years ago, when a basic scientist found a fruit fly on the laboratory wall whose eyes were white instead of the usual red. Years later, researchers discovered that this particular strain of fruit fly had white eyes because one of the fly's genes hadn't worked properly. These fundamental observations touched off an enormously productive field of study. For decades, NIH-supported basic scientists have used the fruit fly model system to make connections between specific genes, normal and abnormal development, and disease in animals, including humans. Some of their achievements are described below.

In the 1970s, researchers pinpointed the genes that control development of body segments, features that make a fruit fly recognizable as an insect. This finding set the stage for other scientists to discover that the same genes control these developmental patterning processes in humans, who do not have recognizable segments. Around that time, fly development researchers studying development in fruit flies began to devote their attention to organs, particularly the eyes, the ovaries, and the testes. Eyes were popular targets because researchers could easily identify important eye genes, since the loss of these genes impairs flies' ability to run toward light. Ovaries and testes intrigued scientists for a different reason. Since only females have ovaries and only males have testes, researchers could study the genes that affect these organs to figure out why these organs develop differently in the two sexes.

One result was the identification of certain genes that, when malfunctioning, make a fly's eyes degenerate, causing blindness. Scientists now know that the genes that cause eye degeneration in flies are also present in humans. Mutations in these human genes are responsible for many cases of macular degeneration, the most common cause of blindness in adults. Detailed studies of the fly genes that cause

eye degeneration should provide clues about how to correct – or even prevent – macular degeneration in people.

Throughout the 1980s and 1990s, fly researchers studied the development of other organs, particularly the heart, the brain, and the respiratory system. They discovered that a growth-promoting molecule called "branchless" directs the proper development of fly respiratory tubules – a network of large and small pipe-like structures that carry oxygen into a fly's body. Recently, scientists found that this growth-prodding protein's ability to regulate the development of small respiratory tubules is controlled by oxygen. Something very similar happens in human embryos, where oxygen-regulated growth factors control the development of the major blood vessels, and later, the development of the smaller capillaries. The striking parallel between the development of the fly respiratory system and the human circulatory system promises to help researchers better understand how blood vessel overgrowth sustains cancer cells, and possibly how enhancing the growth of oxygen-carrying vessels may help treat a variety of forms of heart disease.

One way scientists use fruit flies to study the brain and diseases that impair the function of the central nervous system is to create flies with human disease genes, and then observe what happens to the flies' behaviors. For example, in March 2000, researchers engineered flies with Parkinson's disease, the second most common neurodegenerative disorder in humans. The scientists gave the flies a human gene that provides brain cells with instructions for making a protein called alpha-synuclein. When this gene malfunctions in humans, the protein piles up in brain cells. The scientists discovered that – just like people with Parkinson's disease – flies with the human gene have trouble controlling their movements. The flies also had lots of extra alpha-synuclein in their brain cells. By studying these flies, researchers hope to be able to determine how the alpha-synuclein gene causes brain degeneration. Scientists should also be able to use fruit flies to identify potential new drugs to treat Parkinson's disease.

For decades, scientists have also studied fly brains to figure out why flies – like humans – have daily cycles of activity and night-time rest (circadian rhythms). In the 1970s, researchers discovered that a gene called *period* is the internal alarm clock controlling flies' activity and rest cycles. Since then, scientists have identified many other fly genes that affect circadian rhythms, including a recently discovered gene called *take-out* that may determine when during the day a fly gets hungry. Humans have *period* genes and many – perhaps all – of the other genes that affect flies' daily cycles. By continuing to study circadian rhythm genes in flies, researchers are likely to gain a better understanding of human maladies such as insomnia, jet lag, and perhaps even eating disorders.

Although scientists studying fruit flies have made impressive strides in understanding development, disease, and behavior, much more work lies ahead. Future researchers can look forward to an easier time of figuring out what the thousands of newly identified fruit fly genes do. After nearly two decades of groundwork, scientists finally succeeded in June 2000 in "knocking out" genes in flies, a genetic technique in which researchers get rid of a working gene and see what happens when it is gone. Because of this technical breakthrough, science will undoubtedly witness an explosion of knowledge about fly and human genes in the coming years.

Epidermolysis Bullosa:
A Bedside to Bench to Bedside Story of Discovery

The story of the incredible difference that biomedical research has made for patients with epidermolysis bullosa (EB) has many chapters and it is a dramatically powerful story. The Prologue tells us that EB is a devastating childhood skin disease that meant either a death sentence or a very difficult life for many patients. On hearing this diagnosis, parents of an affected child learned that their child's skin is extremely fragile and even the slightest friction can cause painful blistering. In some severe forms of EB, blisters can cover most of the body and occur in the digestive tract. Often patients with severe EB have wounds that resemble serious burns. One out of every 50,000 infants is born with EB, and they typically face physical, emotional, and financial hardships for themselves and their families.

Chapter 1 begins three decades ago when electron microscopic studies of affected skin were the only means of diagnosis. These studies showed that some patients with scarring (dystrophic) forms of the disease had no anchoring fibrils (a structural component of skin that is, in part, responsible for adherence of the outer epidermis to the inner dermis); others had greatly reduced numbers of anchoring fibrils; while others had wispy or rudimentary anchoring fibrils. As more patients were examined, it became evident that the patients with no or greatly reduced numbers of anchoring fibrils had a recessively inherited form of EB (caused by two defective genes), and those patients with wispy or rudimentary anchoring fibrils had a dominantly inherited form of EB (caused by a single defective gene overriding a normal gene).

Chapter 2 starts almost two decades ago. Clinicians were treating patients with this rare, hereditary blistering disorder of the skin and mucous membranes, but were hampered by the limited choices of effective treatments, a shortage of accurate methods for differentiating different forms of EB, and a lack of understanding of the disease process. In 1981, the NIH organized a research workshop on EB to bring together leading experts with a passion for solving the mystery of EB and offering a better life to afflicted people, as well as others who were outstanding scientists without any knowledge of EB. This interdisciplinary workshop yielded fruitful results as it encouraged a broad range of scientists to study EB.

Chapter 3 of our story belongs to the basic scientists who studied skin from affected patients and normal individuals. Their contributions to solving this mystery actually appear throughout this story. Scientists who studied the basic biology of the skin's basement membrane zone (the layer of skin between the epidermis and the dermis) learned that the major component of the anchoring fibrils (the missing components in the skin of some EB patients) is type VII collagen (collagen is the body’s major structural protein), and its disruption resulted in separation of the epidermis from the dermis. More recently scientists applied powerful tools of molecular biology to identify the specific genes and proteins underlying most of the hereditary forms of EB. Other scientists added another important dimension to

this story of discovery by identifying defects in a human gene for keratin, the main constituent of the outermost layer of the skin, in patients with different forms of EB.

Chapter 4 occurs in concert with chapter 3 and focuses on the establishment of a registry and a repository for tissues of patients with EB, funded by NIH and DEBRA (Dystrophic Epidermolysis Bullous Research Association), the leading voluntary group focused on EB. A major accomplishment of the first registry and repository was the development of diagnostic methods and criteria for the various forms of EB. These provided useful tools for physicians treating patients as well as researchers in the laboratory. NIH supported this registry for 10 years and the key indication of the success of this registry is the fact that they essentially put themselves out of business, as adequate numbers and types of patients were identified. Currently NIH supports an EB Registry Coordinating Center to maintain the existing data base, acquire new patients that would especially benefit from new therapeutic interventions, and perform limited tissue banking and specialized diagnostic studies.

In chapter 5, with the clues and insights gained through research, scientists at a clinical registry site were the first to demonstrate the usefulness of a new technique of cultured skin cells as an approach for wound healing for particular forms of EB. This approach was subsequently commercialized by several companies providing burn care biologic dressings – with ramifications far beyond the small population of patients with EB. Industry was also attracted to this field when patients with EB wounds were used in the premarketing study of mupirocin, a drug that was later approved by the FDA and is now widely marketed as a prescription topical antibiotic for skin infections.

EPILOGUE: A significant difference between the stories of research and the stories we read in literature is that the research stories rarely have a finite and definitive ending. There have been dramatic changes in the lives of patients with particular diseases, but we continue to learn more about the diagnosis, treatment, and prevention of diseases every day. The newest chapters for EB reveal that (1) these studies have provided insight into understanding the underlying abnormalities in internal organ complications in patients with EB and provide the basis for understanding these abnormalities when they occur in individuals without EB. For example, patients with one form of EB have pyloric atresia, in which the lower end of the esophagus or stomach is narrowed and these patients can eat only liquid or pureed foods, sometimes through a feeding tube. Research on EB has revealed the role of an EB gene as well as the function of particular proteins in pyloric atresia. Research on other patients with another form of EB who have late onset muscular dystrophy has resulted in new understandings of this non-skin disease based on the function of this associated protein (plectin) expressed by an EB gene. (2) EB is a prime candidate for gene therapy. The NIH is supporting a research laboratory that is developing vectors and animal and tissue culture models for testing, and plans to conduct a small clinical trial to test gene therapy in EB patients. (3) Skin proteins discovered in the investigations of EB have also been shown to be targets for autoantibodies in a number of autoimmune blistering skin diseases including bullous pemphigoid, herpes gestationis, acquired forms of EB, bullous systemic lupus erythematosus, and circatricial pemphigoid. This has furthered our understanding of these acquired diseases, which

taken together are far more common than EB, as well as furthered our understanding of the normal structure and function of skin. (4) The availability of an animal model for human diseases facilitates study of the disease process. Naturally occurring models exist for some diseases, but not all of them are true representations of the human disease. In the latest research within the last year on animal models, researchers reported that, with molecular genetic technology, it has become possible to introduce into animals the specific defect known to underlie specific human diseases, and investigators have now done this successfully with the dystrophic form of epidermolysis bullosa. The disease is caused by a defect in the gene for type VII collagen. The availability of an animal model that has the same defect as the human disease will enable study of the disease process, and development and testing of potential interventions.

Patients with EB have taught researchers a lot about disease causes as well as basic biology, with ramifications that could never be predicted. Thus, this is truly a bedside to bench to bedside story. Patients with EB still face many challenges, but they are the beneficiaries of many years of sleuthing in laboratories around the country and the passions of researchers determined to understand and conquer this difficult disease. Medical research has made a genuine difference in the lives of patients with EB.

Improving Treatments, Preventing Relapse: Atypical Antipsychotic Medications

Schizophrenia, a disabling, long-term illness that affects an estimated 1 percent of the population worldwide, is among the most severe forms of mental disorder. Through much of the 19th and first half of the 20th centuries, patients with schizophrenia typically were locked away from society. In the 1950s, investigators in France chanced upon a class of medications that proved to have a remarkable calming effect on severely psychotic patients. With the new drugs, called antipsychotic, or neuroleptics, patients long thought to be beyond reach of any intervention short of physical restraint were relieved of such disturbing psychotic symptoms as hallucinations – for example, hearing voices that are not connected to a visible source – and delusions – beliefs that are not subject to reason. Many patients appeared able to re-enter society.

Although the immediate clinical effects were dramatic, researchers did not know how the new compounds acted on the brain to affect behavior. At the NIH and elsewhere in the United States, basic science surged as scientists used these and other psychoactive medications as tools to learn about brain mechanisms in illnesses long thought to be incurable. Soon, however, research and anecdotal experience afforded an unsettling perspective on the overall effects of antipsychotics. Although the medications relieved many frightening, visible symptoms of psychosis, large numbers of patients experienced a range of often severe adverse effects – for example, neurologic and neuromuscular effects called extrapyramidal symptoms, or EPS. These appeared as uncontrollable, or spastic, muscular contractions that resemble the tremors and rigidity of Parkinson's disease. Tardive dyskinesia, a condition involving involuntary movements and tics – most noticeably of the face, lips, and tongue – disproportionately affected patients who took antipsychotic medications for many years. Another drawback was the relatively small impact neuroleptics had on symptoms of social withdrawal and apathy. For patients discharged into community settings, these persistent symptoms proved to be as devastating as outwardly psychotic symptoms, given the manner in which they worked against the goal of re-engaging in the activities of daily life.

Over several decades, researchers sought to fine-tune methods of administering various look-alike antipsychotic medications. Other studies led to the development of very useful community treatment strategies and psychosocial treatments to complement medications. In diagnostic research, a useful refinement was the categorization of "positive" and "negative," or deficit symptoms. Hallucinations, delusions, and disorganized thinking were termed *positive symptoms*, because they represent an addition – albeit an unfavorable one – to normal, healthy behavior. Withdrawal and other symptoms such as loss of motivation to engage in productive behaviors or satisfying social relationships were called *negative symptoms* because they represent a loss of, or deficit in, behavior that was normal for an individual before the illness occurred.

On a parallel track, the opportunity afforded by psychoactive medications to study the cellular and molecular mechanisms of the brain's operations helped to fuel the explosive growth of neuroscience.

Early research findings suggested that the drugs' effectiveness pointed to simple excesses or deficiencies in certain brain chemicals called neurotransmitters; this "single neurotransmitter" interpretation proved to be overly simplistic, but led to an increasingly sophisticated appreciation of the complexity of brain systems in mental disorders.

While these multiple strands of research were being pursued, the Food and Drug Administration approved in 1990 a fundamentally new type of antipsychotic drug, clozapine, for use in the U.S. The clinical advantages of clozapine were clear: a virtual absence of the most troublesome motor, or movement, side effects associated with conventional antipsychotics and a greater degree of efficacy in addressing negative as well as positive symptoms. Clozapine, however, was not side-effect free. In a small percentage (1%) of patients it caused a potentially fatal blood condition: *agranulocytosis*, a loss of the white blood cells that fight infections, so all patients taking clozapine are required to have their blood tested weekly and discontinue the drug if necessary.

NIH-funded grantees and intramural scientists were at the forefront of efforts to evaluate the clinical and cost-effectiveness of clozapine. Findings that the effectiveness of the new compound in reducing need for repeated hospitalizations and length of inpatient stays largely compensated for its expense gave impetus to efforts, largely undertaken by the pharmaceutical industry, to develop other medications – which would be called *atypical* antipsychotics because they have a different therapeutic action than their predecessors – with therapeutic benefits that would meet or exceed those of clozapine, while avoiding serious side effects. The eventual success of industry in this quest would prove to rely heavily on basic NIH funded research.

Initially, the mechanism of action of clozapine was virtually undecipherable. Scientific appreciation of the complex interactions between such neurotransmitters and the specialized molecules called receptors that are found on the surface of neurons and are the sites at which neurotransmitters – and drugs – influence cellular action, was relatively primitive. At that time, scientists had identified only two receptors for the neurotransmitter dopamine, and two for serotonin. Over the past decade, researchers have identified as many as five structurally and functionally distinct receptors for dopamine and 14 for serotonin. These discoveries are playing a critical role in determining the action, or effect, of clozapine; as importantly, they afford specific and increasingly well-characterized, novel targets for drug development research.

Brain imaging also is beginning to play an increasingly prominent role in medication development efforts. Using newly identified radioligands – molecules that bind to the same receptors that medications do – NIH-funded research now underway is using positron emission tomography, or PET scanning, to examine regional differences in how conventional and atypical medications work. This information opens another window on the therapeutic actions of atypical antipsychotics and other medications.

In recent years, the FDA has approved several new atypicals – risperidone (Risperidol), olanzepine (Zyprexa), and quetiapine (Seroquel) – and yet others are in the pipeline. Although first used to treat schizophrenia, the safety and potency of second-generation atypicals has encouraged their use in treating other disorders, including the manic phase of bipolar disorder and behavioral symptoms associated with Alzheimer's disease and other dementias. NIH is currently conducting the Clinical Antipsychotic Trials of Intervention Effectiveness project (CATIE), http://www.catie.unc.edu/, which is evaluating the effectiveness of atypicals in the treatment of schizophrenia and Alzheimer's disease under real world conditions.

The introduction of atypical antipsychotic medications is noteworthy for the complementary roles assumed by NIH on the one hand, and the worldwide pharmaceutical industry on the other. Most critically, evidence that rigorous scientific study of schizophrenia and other severe mental disorders can lead to effective treatments and allow patients to reenter the community has proven to be our most potent tool for crushing the stigma attached to mental disorders while enhancing opportunities for health and improved quality of life for millions of persons.